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ESTIMATION OF QUALITY OF FORAGE CROPS BY ARTIFICIAL RUMEN TECHNIQUES

by

John Malcolm Asplund

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ABSTRACT

Five experiments were conducted to assess the value of the miniature artificial rumen in the estimation of forage quality.

Experiment 1 demonstrated that the dry matter loss and volatile fatty acid production of forages in the artificial rumen were significantly different when forages of obviously different quality were fermented or when inocula from sheep on rations of different quality forages were used.

In Experiment 2, 11 forages of known digestibility were fermented in the miniature artificial rumen by 2 different inocula. The dry matter loss and volatile fatty acid production were determined. Artificial rumen results showed a significant degree of correlation with the dry matter digestibility coefficients of the 11 forages. This correlation was not sufficiently high for reliable prediction. Fermentation time of 18 hours lowered the correlation, while a 48 hour incubation period did not raise it significantly. Washed cell inocula also did not raise the association sufficiently for reliable estimation of forage quality. Correlations were calculated using data for 9 forages, excluding the results of the fermentations of 2 mature *Kochia* samples. These *r* values were highly significant and large enough to indicate a possibility of reliable prediction of forage quality. The validity of discarding the results for the 2 *Kochia* samples is discussed.

Experiment 3 showed that the associations demonstrated in Experiment 2 were repeatable using a different group of forages.

Voluntary dry matter consumption as an index of forage quality is discussed in Experiment 4. It was shown that volatile fatty acid production gave a relatively good indication of voluntary

forage intake. The difference in the effects of different curing procedures on the quality of forages was also apparent.

Rabbit and sheep feeding and digestion trials, and artificial rumen techniques were used in Experiment 5 to determine the effect of fertilization on the feeding value of forages. Despite limitations inherent in the experiments, it was demonstrated that sulfur fertilization of alsike clover grown on a sulfur deficient grey wooded soil in Alberta is effective in increasing the feeding value of the forage.

The general thesis is presented that results obtainable by miniature artificial rumen techniques, while not direct indices of any specific criterion of forage quality, are sufficiently closely associated with the *in vivo* utilization of roughages to be of value in the estimation of forage quality.

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INTRODUCTION

The evaluation of the quality of forages for domestic animals is a problem of considerable economic and scientific importance. This is so because the most abundant source of plant energy is the complex carbohydrate fraction which includes the celluloses and hemicelluloses. These compounds are not made available for use as energy sources by the enzymes secreted by the digestive organs of farm animals, but must be made so by means of fermentation by microorganisms living symbiotically in the fore-stomach or cecum of various species. Ruminants are the most important group of domestic animals through which large quantities of cellulose and hemicellulose are made available for human use. They possess a highly specialized and efficient fore-stomach in which exist ideal conditions for symbiotic fermentation of fiber. The biota of the rumen ferment large quantities of complex carbohydrates and produce numerous compounds that are utilized or eliminated as waste by the host.

Determination of feed quality can be considered as the estimation of the net good in terms of economic use to man per unit of feed. Thus the evaluation of forage quality is vastly influenced by the extent and rate of the symbiotic fermentation of the fiber fraction which comprises a large percent of the total nutrients of forages. An accurate assessment of the total value of a forage is not easy to obtain. The most frequently used method of assay is the feeding or digestion trial, but this technique is expensive, complicated and requires large amounts of feed. Moreover, individual variation can exist between test animals introducing substantial experimental error. The use of proximate analysis gives the advantage of speed, economy and small samples but the application of the results of these analyses to actual biological data is based upon arbitrary hypotheses and is highly

questionable. Results of feeding trials are often at wide variance with results expected on the basis of proximate principles.

Since the development of a practical and reliable artificial rumen by Marston and its simplification and development by later workers, there is strong evidence that a representative rumen fermentation may be carried out in the laboratory. Recent work suggests that the artificial rumen might provide a method of forage evaluation which would be reliable biologically and yet rapid and convenient for laboratory use. This technique could be useful for pilot experiments for digestibility trials, for studies involving large numbers of samples or for assaying where only small samples are available.

This thesis considers the use of the miniature artificial rumen in some aspects of evaluation of forage quality.

REVIEW OF LITERATURE

A. General

The Ruminant Stomach

The ruminant stomach is divided into four distinct compartments; The rumen, reticulum, omasum and abomasum. The first three represent non-glandular enlargements of the embryonic stomach while the fourth is glandular. The whole stomach has a capacity of 25 to 60 gallons in mature cattle depending on the size of the individual. The rumen accounts for about 80% of capacity, the reticulum 5%, the omasum 7 to 8% and the abomasum 8 to 7 percent. The rumen has a dorsal and a ventral sac and communicates with the reticulum over the rumeno-reticular fold. The fermentations in the reticulum are similar to those in the rumen and the two are usually considered together as rumen fermentations. The reticulum communicates through the reticulo-omasal orifice with the omasum which is connected to the abomasum. The exact role of the omasum is not certain. The abomasum is a true glandular stomach whose function is similar to that of a normal monogastric stomach and is connected to the duodenum, Dukes (1947).

Fate of Ingested Feed

Food is ingested by a ruminant rapidly and with only sufficient mastication to insalivate it. This feed, in the form of boluses, is directed into the rumen where it is saturated, macerated and partially fermented. After a meal most of the feed is regurgitated, remasticated, reinsalivated and returned to the rumen. This cycle is called rumination. In the rumen the returned feed is further fermented and then passes through the omasum into the abomasum and duodenum for continued digestion, Dukes (1947).

The rumen fermentation enables the animal to utilize large

quantities of roughages which contain high proportions of otherwise indigestible celluloses and hemicelluloses. Microorganisms in the paunch attack the complex carbohydrate fraction under anaerobic conditions and ferment it with the production of methane, carbon dioxide and volatile fatty acids. The volatile acids are used by the host as a source of energy and the gases are eliminated as waste either by eructation or by absorption into the blood stream and diffusion through the lungs, Dukes (1947).

B. Factors Affecting Rumen Fermentation

The rumen represents an ideal site for fermentation. The temperature remains fairly constant at about 40° C., Burroughs et al. (1950). This is a little higher than usual body temperature and is probably due to heat formed during fermentation. The pH varies only slightly in spite of large quantities of acid produced. Huffman (1956) reported an average pH of 7.3 before feeding and 6.5 after feeding for cattle. However, values as low as 5.7 have been reported for sheep on certain rations, Myburgh and Quin (1943). There is a constant generous supply of mineral buffer in the form of saliva and provision for almost continuous supply of substrate and removal of metabolic wastes. As a result the rumen contains a very vigorous and extremely numerous population of microorganisms. Bacteria vary in number depending upon many factors, but they number in the billions per gram of rumen fluid. Protozoa also vary in number and type with the diet and age of the animal.

Because of the large variety of species of microorganisms that live in the soil and because soil is introduced into the rumen in feed, a multitude of species can exist in the rumen but such transient organisms cannot be considered as the representative rumen flora. There are, however, certain basic types that can be considered true rumen

organisms. Gall et al. (1951a) have advanced five criteria for judging true rumen organisms. These are:

(a) anaerobiosis,
(b) present in numbers of at least 1,000,000 per g. of fresh rumen contents,
(c) isolation of a similar type at least 10 times from at least two animals,
(d) isolation from animals in at least two geographical locations,
(e) production of end products found in the rumen from substrates found in the rumen.

Several species fulfilling these criteria have been isolated and described. Among these are: curved rods, Huhtanen et al. (1953a); lactate users, Huhtanen et al. (1953b); species of Bacilli, Clostridia, Corynebacteria and Micrococci, Appleby (1955); Bacteriodes succinogenes, Bryant et al. (1954), and several miscellaneous types, Huhtanen et al. (1953a, 1953b), Gall et al. (1951a). The exact role of each of these species is not known and much more work is needed to understand the place of each in the highly complicated population of rumen fluid. Many workers have considered the mixed culture as a unit using cellulose digestion and urea utilization as the criteria of the activity of this unit.

Nutritive Requirements of Rumen Bacteria

Energy: Early investigation showed that the addition of readily available energy to the ration depressed cellulose digestion *in vivo*. Burroughs et al. (1949a) found that starch added to rations of corn cobs or alfalfa depressed the digestibility of the dry matter. Maynard (1947) stated:

> "It has been shown......that the addition of easily digestible carbohydrates such as starch, cane sugar, or molasses to the ration of cattle reduces the digestibility of the fiber and this observation has been explained on the ground that the bacteria attack the simpler carbohydrates by preference."

In some cases, however, easily digestible carbohydrates have been shown to increase fiber digestion. Burroughs et al. (1949b)

observed that protein as skim milk increased roughage digestion when fed with starch and that no increase was obtained when skim milk alone was used as a supplement. Arias *et al*. (1951) reported that small amounts of readily available carbohydrates increase cellulose digestion while large amounts depress it. Utilization of urea is always increased by available energy. Belasco (1956) found that starch aided cellulose digestion but dextrose did not. MacLeod *et al*. (1954) found that low amounts of glucose aided cellulose digestion when fed with supplements of certain fisheries by-products. These reports indicate that a small amount of readily available energy is required by the microflora of the rumen in order to initiate the digestion of holocelluloses, but that once the fermentation is begun, excess amounts of available energy are not needed. The difference between the activity of starch and dextrose in this regard is perhaps due to differences in availability of energy because the type of carbohydrate that supplies available energy greatly affects digestion. Belasco (1956) reported that starch is the most effective carbohydrate in urea utilization, while cellulose is next and xylan and pectin last. He also found that the carbohydrate affects the magnitude and type of volatile fatty acid production. Johnson *et al*. (1956) reported that the form of energy drastically affects the types of bacteria and protozoa present in rumen fluid. A high starch ration produces a decidedly different population than does one of high cellulose. The fermentation pattern also varies with the type of carbohydrate fermented. The cellulose fraction of timothy and alfalfa hay underwent a uniform decrease in rate of fermentation with time *in vitro*, while the breakdown of wood or cotton linters retarded sharply after initially high rates, Salsbury *et al*. (1955).

Protein: *In vivo* studies by Burroughs *et al*. (1949c) showed

that soybean oil meal added to ground corn cobs appreciably increased the digestibility of the dry matter of the ration. Belasco (1954a, 1954b) reported that urea was superior to protein supplements as a source of available nitrogen for rumen microorganisms. He showed that the use of 1:1 urea, protein supplement mixtures was also effective and stated that the availability of nitrogen was of great importance. An increase in urea as the sole source of nitrogen gave an increase in cellulose digestion up to a certain point where excess urea produced toxic ammonia and inhibited fermentation. He also reported that urea supplementation produces higher amounts of volatile fatty acids than does supplementation with other protein rich feeds. Woodhouse _et al_. (1955) stated that the amount and nature of protein in the feed greatly affects the biota of the rumen.

Additional nitrogen in the form of urea or soybean oil meal increased the synthesis of riboflavin, nicotinic and pantothenic acid by rumen microorganisms, Hollis _et al_. (1954). Ample available nitrogen seems to be a very important factor in almost all phases of the activity of the rumen microflora.

Fat: The effect of fat on the digestion of holocelluloses may become a very important question as rations tend to become higher in energy. It has been found that corn oil and lard lowered cellulose digestion by 40 to 94% _in vitro_, and depressed protein digestion and fatty acid production _in vivo_ and _in vitro_. There was a decided change in odor and color of the rumen fluid and the population of bacteria changed to yield fewer small rods, more small cocci and more aerobes, Brooks _et al_. (1954).

Minerals: Gall _et al_. (1949)found that in cobalt-deficient

sheep the numbers of bacteria were reduced and the proportions of type-cultures were changed. This was not due primarily to the reduced feed intake of the deficient animals. Bohstedt (1949) stated that cobalt is required by rumen bacteria; however, Salsbury et al. (1956) reported that high levels of cobalt depress cellulose digestion. Twelve p.p.m. depressed cellulose digestion somewhat while 100 p.p.m. depressed it considerably. Rumen fluid from steers fed a high cobalt ration had an impaired ability to ferment fiber. Whether cobalt is in itself toxic, or whether it competitively inhibits other mineral effects, is not known.

Gall et al. (1951b) reported that the presence or absence of sulfur in a purified ration greatly influenced the types and number of bacteria. Burroughs et al. (1951) reported that, in addition to the sodium, potassium, calcium, magnesium, chlorine and sulfur added in the nutrient buffer, rumen organisms in vitro required phosphorus and iron. Using o-phenanthroline as a chelating agent, McNaught et al. (1949) determined that 1 p.p.m. iron was needed to support normal protein synthesis in rumen fluid.

Alfalfa ash is known to have an effect on rumen microorganisms. Hollis et al. (1954) stated that alfalfa ash added to corn cobs increased the synthesis of riboflavin, nicotinic acid and pantothenic acid by bacteria fermenting the ration. The adverse effects of high fat are somewhat corrected by the use of alfalfa ash, Brooks et al. (1954). Whether the growth factor in alfalfa ash is a simple mineral element or a more complex compound is not known.

Unidentified Factors: It has been observed that cell-free rumen fluid contains some factor(s) necessary to the normal activity of the rumen biota. This factor(s) is not supplied by commercial media

devised for nutritionally fastidious bacteria, Bryant *et al*. (1954).

Bentley *et al*. (1954)reported that the acid distillate and the alkaline residue of rumen fluid contained a factor which stimulated the cellulolytic activity of rumen bacteria. This suggested that at least part of the factor(s) might be in the volatile fatty acid fraction. Valeric, iso-butyric, iso-valeric and caproic acids did have some beneficial effects on cellulose digestion, and work by Johnson *et al*. (1956) showed that some rumen species were affected by 5 and 6 carbon fatty acids. However, Huhtanen *et al*. (1956) reported that valeric and iso-valeric acid did not increase cellulose digestion in trials *in vitro*, while Hungate *et al*. (1956) found that valeric and iso-valeric acids did not increase straw utilization in steers but did increase feed consumption and appetite. In view of these conflicting results it is evident that some other factors must be involved.

Hungate (1955) stated that a branched and an unbranched short chain fatty acid were essential in relatively high concentrations for one cellulolytic species, but that some other species required a factor in rumen fluid that was not a fatty acid. There is a factor(s) in active yeast extracts, dried distillers' solubles, alfalfa meal, autolyzed yeast, casein hydrolysate, whale solubles, herring solubles, herring stickwater and halibut muscle hydrolysate that stimulates cellulose digestion by rumen bacteria, MacLeod *et al*. (1954), Bentley *et al*. (1954), Dehority *et al*. (1957), which has been identified with the amino acid fraction of these materials. MacLeod *et al*. (1954) found that a mixture of 18 amino acids was more effective in stimulation of cellulose digestion than was whale solubles. Bentley *et al*. (1954) reported that amino acids, especially valine, were very active cellulolytic

stimulators. Proline, valine and the leucines are reported to be the most active amino acids in this respect, Dehority et al. (1957).

Other materials have been found to be active. Johnson et al. (1956) found that some species of rumen bacteria are affected by biotin, p-aminobenzoic acid and vitamin B_{12}. Bryant et al. (1954) reported that a factor in rumen fluid which was not a B vitamin, amino acid, peptide, purine, pyrimidine or mineral, not in several materials used to grow nutritionally fastidious bacteria or in extracts of alfalfa meal or bovine feces, was found to be necessary for normal activity of one common rumen species.

None of these materials seems to supply the complete answer. McNeill et al. (1954), after trying several different factors stated:

> "No materials have been found which possess the same stimulatory effect for the over-all rumen population as does rumen fluid."

This corroborates the statement of Baker, quoted by Thaysen (1945) that all physiological types of bacteria in the rumen will not grow on a given artificial medium.

The Effects of Antibiotics on Rumen Microorganisms

The effect of antibiotics on the activity of rumen bacteria has been investigated because these compounds are now extensively fed to many classes of livestock. Chlortetracycline and oxytetracycline have been found to reduce significantly the digestion of cellulose and other carbohydrates in vitro, Huhtanen et al. (1956), Jurtshuk et al. (1954). Streptomycin, penicillin and chlormycetin at high concentrations also inhibited cellulose utilization in vitro, Wasserman et al. (1952). Antibiotics produced marked changes in artificial rumen populations but the populations adapted very rapidly and returned to normal, Lodge et al. (1956), Luther et al. (1954). Moreover, the rumen fluid from antibiotic-

fed animals exhibited normal activity, Hungate et al. (1955), Huhtanen et al (1956). These results indicate that rumen microorganisms, especially in vivo, are able to adjust to the levels of antibiotics usually fed to stock.

Miscellaneous Factors in Cellulose Digestion

Cason et al. (1955) reported that aqueous extracts of several common feeds, both roughages and concentrates, possess considerable cellulolytic activity. This self-contained cellulolytic ability perhaps contributes greatly to cellulose digestion in vivo. Baumgardt et al. (1956) also observed considerable dry matter loss in fermentations of feed using only water as an inoculum instead of rumen fluid. The actual extent of this endogenous cellulolysis or its primary cause is not known.

The reticulo-rumen is not the only site of cellulose digestion in the ruminant. Conrad et al. (1956) proposed a large amount of activity in the cecum. Hale et al. (1947) estimated this activity as 11.6% of cellulose and 9.5% of protein digested in the cecum. This cecal fermentation, while not extensive, may give rise to anomalies in attempting to apply in vitro rumen fermentations to situations in vivo.

C. Production of Volatile Fatty Acids

As early as 1884, Tappeiner, as cited by Marston (1939), observed that one of the main end products of cellulose fermentation by rumen fluid in vitro was volatile fatty acid. These short chain fatty acids have since become recognized as the chief product formed in the fermentation of cellulose by rumen microorganisms.

The relative amounts of the short chain acids produced in the rumen of sheep on a ration of wheaten hay were formic 1%, acetic 68%, propionic 18%, n butyric 9%, iso-butyric 0.7%, valeric 2.6%,

caproic 0.7% and a trace of heptoic, Gray et al. (1951a). This is characteristic of animals on a diet of roughage. Addition of casein or other protein supplement increased the percentage of butyric acid, Gray et al. (1952). Davis et al. (1957) reported that there was an increase in the level of all the higher volatile fatty acids in the rumina of cows fed high protein rations. There was a decrease in the percentage of acetate and an increase in butyrate.

Proof by Barcroft et al. (1944) that the volatile fatty acids are absorbed through the wall of the rumen confirmed the belief held by many workers that these acids are an important source of energy to the animal. This absorption, which may also occur to some extent in the omasum, is quite efficient. Conrad et al. (1956)reported that volatile fatty acids introduced into the reticulo-rumen of young calves were almost completely absorbed. Each acid is absorbed at a different rate. Flatt et al. (1956) stated that the rates of absorption are: butyric 2.66 g. per hour, propionic 2.26 g. per hour, acetic 1.69 g. per hour for young dairy calves.

Gray et al. (1950) measured the fatty acid production in vitro and in vivo for more mature animals. He gave the following data:

VOLATILE FATTY ACID PRESENT IN RUMEN FLUID

		Acetic %	Propionic %	Butyric %
Wheaten Hay	in vitro	46	41	13
	in vivo	71	17	12
Lucerne	in vitro	59	27	14
	in vivo	70	14	16

The low percentage of propionic acid measured in vivo as compared to that in vitro would indicate that this acid is more rapidly

absorbed than either acetic or butyric acid. Thus, in mature ruminants, propionic acid seems to be the most rapidly absorbed of the volatile fatty acids.

Workers now agree that the fatty acid produced in the rumen is an important source of energy to the animal but estimates of the actual proportion of energy supplied differ widely. Phillipson (1948) calculated that this fraction can supply 46 to 120% of the fasting requirements of a Holstein cow. Carroll and Hungate (1954) estimated that the lower fatty acids supply about 70% of the total energy requirements of the animal, but Emery *et al*. (1956) have postulated that only 3 to 13% can be supplied by this source. Most authors agree with the higher figures and, indeed, if the end product of cellulose digestion is volatile fatty acid, only the higher figures indicate typical ruminant efficiency in the use of roughages. The rapid absorption gives rumen fluid a deceptively low concentration of fatty acid and calculations on the basis of actual concentration in the rumen will probably be low. *In vitro* techniques give a more complete picture of total acid produced.

The main substrate from which volatile fatty acid is produced is the carbohydrate fraction of the feed. Acetic, butyric and propionic acids are produced from starch, maltose, glucose and cellulose, while acetic and propionic acid are produced from pyruvate and lactate. Maltate,αketoglutarate, succinate, aspartate, malonate and glutamate all produced some volatile fatty acid, but alanine produced none, Hershberger et al. (1956).

Various transformations occur between the different acids. Using C^{14} labelled groups, Gray *et al*. (1951a) showed that acetic acid was transformed to propionic and butyric acids in considerable quantities.

In all probability large amounts of formic acid are produced, but this compound is very quickly decomposed to produce methane, Carroll and **Hungate** (1955). Lactic acid is also formed in considerable quantities. Robinson et al. (1955) reported that rumen bacteria produce lactic acid from glucose, fructose and maltose, but not from xylose or arabinose, and Waldo et al. (1955) found that grain and glucose give a larger quantity of lactate than do starch or pyruvic acid. However, lactic acid is seldom present in large quantities because it is readily transformed. Hershberger et al. (1956) stated that acetic and propionic acid were formed from lactate. Huhtanen and Gall (1953b) described two lactate-using species of bacteria.

Estimation of Volatile Fatty Acids

Fatty acids in solution may be separated by steam distillation in the presence of a strong acid because if the salts of a weak acid are in solution with a non-volatile strong acid, they exist largely in an undissociated state and may be volatilized. Steam distillation is used because the acids are relatively non-volatile. Neish (1952) described a method of steam distillation which gave quantitative recovery of the lower monocarboxylic acids. Pigden (1955b) demonstrated adequate recovery of acetic, propionic and butyric acids by steam distillation.

Because for members of a homologous series the partition coefficients between a variety of organic solvents and water increase rapidly as the number of carbon atoms increases, the separation of fatty acids by simple partition has been accomplished by several techniques. Elsden (1946) described a method using a column of silica gel with a brom cresol indicator. Moyle et al. (1948) modified this

procedure and used three buffered columns of silica gel without an indicator. In both cases the solvent was chloroform and *n* butanol. The method of Ramsay and Patterson (1945) is the official A.O.A.C. method for the determination of volatile fatty acids in various foodstuffs. This technique employs a silicic acid column and chloroform-*n* butanol solvent. Neish (1952) described a method using acid-washed celite in the column and a chloroform-*n* butanol solvent. Keeney (1955) described a method using a silicic acid column with a brom cresol green indicator and hexane-*n* butanol solvent which is used directly on rumen fluid.

James and Martin (1952) have successfully used gas-liquid partition in the separation of lower fatty acids, and Sato *et al*. (1947) have used counter current distribution techniques in the separation. Thus there is a wide variety of methods, the choice of which depends on the nature of the sample, the equipment available and the accuracy desired.

D. Gas Production in the Rumen

During anaerobic fermentation in the rumen, large quantities of gas are produced. The rumen is essentially anaerobic and the oxygen that is ingested in the food is very quickly utilized by aerobic organisms in the paunch or is displaced by the gases which are rapidly evolved. Kleiber *et al*. (1943) gave the analysis of these gases as: 67% CO_2; 26% CH_4; 7% $N_2 + H_2$; 0.1% H_2S and less than 1% O_2. The carbon dioxide and methane are the most abundant gases and also the most vital to nutritional investigation because they represent a portion of the energy metabolism of the rumen. Carbon dioxide comes from two main sources, the metabolism of carbohydrate and the other food materials, and from the carbonate and bicarbonate of saliva.

This gas is produced in large amounts but much of it is rapidly absorbed through the rumen wall, Kleiber (1955).

Methane is produced by the reduction of carbon dioxide by means of a very efficient hydrogenase systen. The carbon dioxide is reduced by hydrogen from the decomposition of formate. This reduction is more rapid than the formate breakdown and therefore only small amounts of hydrogen accumulate, Carroll and Hungate (1955). This was demonstrated by McNeill _et al_. (1956) who showed that rumen bacteria in the presence of hydrogen acceptors produced less methane and more carbon dioxide,while in the presence of hydrogen donors, the methane was increased and the carbon dioxide reduced. McNeill _et al_. (1955) also reported that no carbon dioxide was isolated from rumen fermentation in a hydrogen atmosphere. As methane production is almost constant at 4.2 to 4.8 g. per 100 g. digested carbohydrate, Kleiber (1955), and because hydrogen is found only in traces in the rumen, it would appear likely that methane production depends very largely on the decomposition of formate which provides molecular hydrogen for methane synthesis. Methane can come from other sources but the main production is by reduction of carbon dioxide, Carroll and Hungate (1955).

Feeds vary in the amount of gas produced from them by rumen bacteria. Added sugar increased carbon dioxide production _in vitro_ and total gas production _in vivo_ and there was some evidence that added carbohydrate will also raise methane production, Kleiber (1955). Pigden (1956) observed that high quality forages fermented more rapidly and produced more gas than those of low quality, and Hungate _et al_. (1955) have used manometric measurement of gas evolved from rumen cultures as an index of the activity of such cultures.

E. The Artificial Rumen

Tappeiner (1884) as cited by Marston (1948) gave one of the earliest reports of fermentations in vitro using rumen microorganisms. He incubated paper pulp and cotton with rumen fluid and reported that the cellulose disappeared with the formation of lower fatty acids. This work was almost forgotten for many years until Marston (1948) devised an artificial rumen in an attempt to duplicate the actual conditions in the paunch. His device included aeration with nitrogen for anaerobiosis, constant temperature, pH control by an inorganic buffer and stirring. Inoculum was rumen fluid from freshly slaughtered sheep. Because the rapid production of acids necessitates careful pH control, Louw et al. (1949) prepared an artificial rumen similar to that of Marston (1948), but carried out the fermentation inside a semi-permeable membrane from which the acid produced could diffuse. Burroughs et al. (1950) developed a simple, practical apparatus which could be used for routine laboratory work. This device had provisions for constant aeration with carbon dioxide, manual pH control with sodium carbonate and an all-glass container. This apparatus was simplified even further by Gray et al. (1951b) who found that ammonium carbonate effected adequate pH control for the whole fermentation period, thus eliminating the manual pH adjustment. Hungate et al. (1955) have used a Warburg respiration apparatus to measure gas production in vitro. Carroll and Hungate (1954) have also used a flask, sealed and incubated under anaerobic conditions, to make some measurements of rumen fermentations.

Huhtanen et al. (1952, 1954) described a miniature artificial rumen using a semi-permeable membrane containing the fermentation mixture

suspended in a mineral buffer similar to sheep saliva, McDougall (1948). This is an extremely simple apparatus and can be used for large numbers of routine samples.

Inoculum: In early designs of the artificial rumen, massive inocula of rumen fluid were used. Burroughs *et al*. (1950) used 40%, i.e. 140 ml. rumen fluid in 350 ml. of fermentation mixture, while Gray *et al*. (1951b) used the same volume of undiluted rumen fluid. Huhtanen *et al*. (1954) used a comparatively small inoculum of only 10 ml. of undiluted rumen fluid. It became apparent, however, that the material carried in this fluid was a serious source of error. Undigested feed particles and soluble fermentation products might easily confound results by supplying unknown amounts of protein, energy, etc. Cheng *et al*. (1955) washed the bacterial cells by centrifugation and re-suspended them in a buffer solution. These washed cells were used as inoculum and gave quite satisfactory results. Although there is some question as to the effect of removal of protozoa and the growth factors contained in rumen fluid, many workers are using washed cells from rumen fluid because this method removes such a large source of error.

Validity of the Artificial Rumen Technique: It is of prime importance to determine how valid the results obtained in artificial rumena are in their application to actual fermentations *in vivo*. Hungate (1955) stated that *in vitro* fermentations were reliable up to 24 hours after which the protozoa die and aerobic and putrefactive organisms increase in numbers. Gall and Glaws (1951) cited by Pigden (1955b) demonstrated that fermentations carried out in semi-permeable membranes retained more typical populations at the

end of fermentation than did those in all-glass reaction vessels. However, Burroughs (1953) remarked that extensive changes can occur in rumen populations without appreciably affecting cellulose digestion.

Warner (1956) gave three criteria for establishing the validity of *in vitro* fermentations by rumen biota:

1. The maintenance of numbers and normal appearance of bacteria, selenomonads and protozoa;
2. the maintenance of normal rates of digestion of cellulose, starch and protein with normal interactions;
3. the ability to predict quantitative results *in vivo*.

Because the rumen population is a dynamic factor, it would appear that the latter two criteria are the more valid. Huhtanen *et al*. (1954) stated:

> "Rumen digestion depends almost entirely upon the microorganisms which are present in the rumen. Therefore, in order for the results obtained from any artificial rumen to be significant, the apparatus must be capable of maintaining true rumen organisms during the whole fermentation period in approximately the numbers in which they are usually found in the rumen. The miniature artificial rumen has been shown to be capable of maintaining the rumen bacteria that could be grown and identified by methods previously reported during the period of active fermentations in approximately the same proportions as they are found in the rumen...... The protozoa were still motile at the end of the fermentation period."

G. Evaluation of Forage Quality Using Artificial Rumen Techniques.

Because the extent of forage utilization depends upon the digestion of the fiber fraction and because this digestion takes place mainly in the rumen, it is very probable that a satisfactory assessment of rumen fermentation would be a large factor in the determination of the quality of a forage. Most investigators have carried out fermentations *in vitro* using a common substrate and testing the effects of

additives. Huhtanen et al. (1954) used the disappearance of carbohydrate as measured by color production with anthrone as an index of the extent of fiber digestion. Pigden (1955b) fermented several feeds of known digestibility in the artificial rumen described by Burroughs et al. (1950) and, using anthrone carbohydrate disappearance as an index, found a significant correlation between dry matter digestibility and carbohydrate digestion. Anderson et al. (1956) used cellulose digestion as an index of the availability of phosphorus in various commonly fed phosphorus supplements. Baumgardt and Hill (1956) have measured the loss in weight of dry matter from the digestion sacs of the artificial rumen of Huhtanen et al. (1954) as an index of extent of digestion. Other evaluations of extent of digestions include fatty acid production, Hershberger et al. (1956), and gas production, Hungate et al. (1955). It is possible that one of these methods, properly applied, might give valuable information concerning the feeding value of forages.

EXPERIMENTAL PROCEDURE

A. The Artificial Rumen Technique

Inoculum

Source: Prior to September, 1956, rumen fluid was obtained from freshly slaughtered sheep. In August, 1956, four mature Suffolk wethers were fitted with permanent plastic cannulae by Dr. J. G. O'Donoghue using the method described by Dougherty (1955), and Phillipson *et al*. (1939). Scar tissue formed around the interior end of the cannulae making them extremely air tight and satisfactory. In February, 1957, when one of these sheep died, two Romnelet yearling ewes were fitted with rumen cannulae to serve as additional sources of inoculum. Rumen fluid was withdrawn through a tube into a suction flask attached to a water pump and was used as soon after withdrawal as possible. Inoculum was collected 3 to 4 hours after the sheep had been fed.

Preparation: Unwashed inoculum was prepared by filtering freshly obtained rumen liquor through two layers of cheesecloth. This material was used in several experiments but it varied greatly as to the amount of dry matter contained.

Washed cells were prepared using the method of Cheng *et al*. (1955). Filtered rumen fluid was centrifuged in a MSE medium centrifuge, using 100 ml. pyrex tubes, at 1000 r.p.m. for 3 to 5 minutes to remove undigested food particles and protozoa. The supernatent was then centrifuged at a speed of 3000 r.p.m. for 35 minutes. The resulting sediment was resuspended in a phosphate buffer of pH 7.0 and again centrifuged for 35 minutes at 3000 r.p.m. The sediment from this operation was suspended in a volume of nutrient saliva buffer equal to one-half the original volume of rumen fluid. This suspension was used as

inoculum for techniques employing washed cells.

The Artificial Rumen

Design: The miniature artificial rumen of Huhtanen *et al*. (1954) was chosen because of its simplicity of design and because several units may be fermented at once. The use of the semi-permeable sac is advantageous in the collection and determination of volatile fatty acids.

The sac is a 6 1/2 inch piece of dialysis tubing which is tied securely at one end. Five hundred mg. of forage as a substrate is placed in the sac and 10 ml. of inoculum is added. The contents are gently kneaded until they are thoroughly mixed and then the sac is suspended in a 4 oz. screw cap jar which contains the synthetic saliva buffer previously warmed to 37.5° C. The sac is secured by catching the open end between the rim of the jar and the lid so that a small part of the sac remains outside the jar. The lid is then screwed in place and the unit is placed in an incubator at 37.5° C. for the desired length of time. It is often desirable to open the lid to release the gas pressure after about 3 hours of fermentation to prevent bursting of the sac. The contents are mixed by gently shaking the jar at the 3rd and 20th hours of fermentation. If washed cell inoculum is used it is not necessary to remove the lid to release gas.

Buffer: The mineral buffer used is synthetic saliva of McDougall (1948). The composition of the buffer in g. per liter of distilled water is $NaHCO_3$, 9.8; Na_2HPO_4, 3.7; KCL, 0.57; NaCl, 0.47; $CaCl_2$, 0.04; and $MgSO_4 \cdot 7H_2O$, 0.12. Carbon dioxide is bubbled vigorously through the solution until the pH is 6.6 to 7.0 Thecloudy solution usually clears at this slightly acid pH.

Type of Sac: The Nojax tubing described by Huhtanen et al. (1954) (Visking Corp., Chicago, Ill. and designated as Nojax cellulose casing 24/32) was satisfactory in most cases. However, the sacs tended to weaken during fermentations which extended beyond 48 hours. Two other types, cellophane dialysis tubing (no. 29070 obtained from Canadian Laboratory Supplies) and cellulose acetate tubing (obtained from Aloe Scientific, St. Louis, Mo.) were tried but both came as tightly rolled, flat tubes which were weak along the crease and as a result they burst easily under even slight pressure. Although the latter tubing was not digested in fermentations up to 96 hours, its lack of strength made it impractical for routine use.

Attempted Modifications of Huhtanen Technique: During the manual release of gas in the original Huhtanen technique, considerable care must be exercised to avoid errors caused by spillage of the buffer solution. An attempt was made to release gas automatically by means of a bunsen valve. This consisted of a piece of rubber tubing plugged at one end with a glass rod and with a longitudinal slit to release gas but prevent air from entering. An Erlenmeyer flask was used instead of a 4 oz. jar and the valve was installed in the rubber stopper. The valve was ineffective when the open end of the digestion sac was caught between the stopper and the edge of the flask. Perforation above the level of liquid in the sac provided for adequate release of gas, but mixing of buffer and fermentation mixture frequently occurred. When the mouth of the sac was tied around the lower part of the valve, gas release was normal, but extreme frothing occurred in the digestion solution and considerable material was forced up into the valve. Release of gas by this method was abondoned as impractical.

Pigden (1956) noticed that fermentation was initiated more rapidly in feeds of high than in those of low quality, and suggested that possibly more gas might be produced by high quality forages. It was attempted, therefore, to devise some method of measuring the rate of gas production during fermentation. Collection of gas was attempted in several different ways. It was hoped to be able to pass the gas through standard sodium hydroxide so that, after addition of barium chloride, the amount of carbon dioxide could be determined, but in all cases the frothing of the fermentation material forced solids into the gas collection apparatus. Several devices to provide adequate counter pressure and to trap the solid material were frequently, but not reliably, effective. It was felt that the development of a suitable apparatus for quantitative collection of gases was beyond the scope of the project and was abandoned because of lack of time and facilities. Because of the difficulties involved in attempted modifications, the method of Huhtanen *et al*. (1954) was used essentially as published.

B. Dry Matter Loss

Baumgardt and Hill (1956) reported that they obtained very reproducible results measuring the loss in dry matter weight after fermentation by the method of Huhtanen *et al*. (1954). Their method was adapted to the present trials and gave very valuable pilot information.

Procedure: Digestion sacs and finely ground forage samples were dried in a vacuum oven for 12 hours at 65°C. and 20 lbs. vacuum. The sacs were then weighed and 500 mg. of forage was placed in each one. These sacs were inoculated and incubated in the miniature artificial rumen. At the end of digestion the sacs were removed, washed

with distilled water to remove any buffer solution and dialyzed in tap water at 12 to 14° C. for 24 hours. After dialysis they were dried by a fan at room temperature and then in a vacuum oven for 12 hours at 65° C. and 20 lbs. vacuum. Inoculum control sac was prepared by placing 10 ml. of inoculum in a weighed sac, adding 2 to 3 drops 40% formaldehyde and refrigerating in a jar of saliva buffer. This sac was dialyzed and dried with the other sacs. The sacs were dried to constant weight and the loss in weight determined.

An example of the method of calculation of percent loss of dry matter is as follows:

Tare weight of sac	= 0.2698 g.
Weight of forage	= 0.5000 g.
Dry weight after digestion and dialysis of sac and forage	= 0.6120 g.
Tare weight of sac for inoculum control	= 0.2587 g.
Dry weight after refrigeration and dialysis of inoculum control sac	= 0.2899 g.
Check value = 0.2899 g. – 0.2587 g.	= 0.0312 g.

$$\frac{(0.2698 + 0.5000 + 0.0312 - 0.6120) \times 100}{0.5000} = 37.80\% \text{ loss of dry matter}$$

Experiments were conducted in order to test the reliability of this method. The results of one such trial using 3 substrates and 2 inocula in triplicate are given in Table I.

TABLE I

DRY MATTER LOSS DURING FERMENTATIONS OF THREE FORAGES BY INOCULA FROM SHEEP FED TWO DIFFERENT RATIONS

	Inoculum	
	A	B
Forage	Average % D.M.L.	Average % D.M.L.
1	51.0	43.0
2	44.0	34.8
3	30.1	23.5

The standard deviation associated with variation between triplicates was 1.53%. The differences between substrates and between inocula were highly significant ($P < .01$) so that this technique was considered as one which satisfactorily demonstrated differences between the fermentations of different forages and in the activity of inocula from different sources and was adequately reproducible. It was used routinely in connection with all fermentations.

C. Volatile Fatty Acids

Steam Distillation

The short chain monocarboxylic acids can be volatilized from strongly acid solution by steam distillation, Neish (1952). Ordinary micro-Kjeldahl apparatus for steam distillation is not satisfactory because a large volume must be distilled and solid material must be introduced into the reaction vessel. The apparatus must provide for heating of the reaction flask and for introduction of a fairly large amount of solid material.

The equipment used in these experiments is an adaption of that described by Neish (1950). It consists of a steam generator, a steam trap, a reaction vessel, a steam trap and a condenser. The reaction vessel is a 250 ml. wide-mouth Florence flask with a three-hole stopper, one hole for steam inlet, one for outlet and one connected to a funnel for flushing the flask. The reaction flask is heated by a Bunsen burner. To the flask are added 16 g. of $MgSO_4.4H_2O$ and 2 ml. concentrated H_2SO_4 and then 25 ml. of sample is quickly pipetted into the mixture and the flask closed. Steam is introduced into the mixture and when the reaction volume reaches about 100 ml. it is kept constant by adjusting the flame under the flask. Distillate

is collected in 50 ml. graduated cylinders and each 50 ml. is titrated separately until 250 ml. has been distilled. The distillation flask is then flushed and a new sample introduced. The distillate is titrated with carbonate-free alcoholic KOH using thymol blue or cresol red indicator.

Separation and Estimation of Volatile Fatty Acids

It was necessary to select or devise a technique which would suit the particular requirements of the artificial rumen method. Such a technique had to provide for separation and estimation of at least C_2 to C_5 volatile fatty acids from a fairly dilute solution of artificial saliva buffer. Several techniques were tried which employed partition chromatography. The method of Elsden (1946) gave separations of only C_1 to C_4 fatty acids and the specialized equipment of James and Martin (1952) and Sato et al. (1947) was not available. The separation described by Neish (1952) was tried and gave fairly good recoveries of pure acid but the necessity of fraction collection made it impossible to run more than one assay at a time.

The method found most suitable to the conditions of the experiment is described by Keeney (1955). In this chromatographic technique an indicator is used which permits visual following of the movement of each acid band so that several columns may be run at once. The time consuming step of steam distillation is eliminated and the sample is analyzed without undergoing rigorous treatment. This method was entirely satisfactory.

Procedure

Preparation of Sample: The buffer solution in the jar of the artificial rumen after the fermentation sac had been washed and removed

was transferred quantitatively to a 600 ml. beaker and thence to a 250 ml. volumetric flask. It was observed that considerable leakage occurred around the protruding end of the fermentation sac. This was a serious source of error in earlier trials. This error was eliminated by placing a watch glass under the jar. Escaping buffer rapidly lost CO_2 and was sufficiently alkaline to prevent the fatty acid present from volatilizing. The outside of the jar and the watch glass were carefully washed into the 600 ml. beaker and these washings were added to the main body of the buffer. This washing was more effective after the jars had been refrigerated for some time. The buffer and washings were made to volume with distilled water and a 50 ml. aliquot was transferred to a 100 ml. beaker. Alkalinity was insured by the addition of 0.5 ml. of 1 N KOH, the beaker was covered with a watch glass and evaporated to dryness on a steam bath.

Standard Base: Aqueous $Ba(OH)_2$ has several disadvantages which make it impractical for use with this method. A saturated solution is approximately 0.01 N which is too low to produce a sharp end point. Small amounts of CO_2 greatly affect the normality of this base. Aqueous base is inconvenient to use with the organic solvents employed in the chromatographic technique.

The most satisfactory standard alkali for the purposes of this technique was 0.1 N alcoholic KOH. This was prepared by adding the required amount of freshly filtered 1:1 by weight solution of KOH to the proper volume of ethanol which had been refluxed for one hour and stored in a container protected from CO_2 by Ascarite and soda lime. A small amount of $BaCl_2$ was added and the solution allowed to stand in a carbonate-protected Winchester for 24 to 48 hours before

standardization. The base was used in a closed system with adequate protection from CO_2.

Chromatographic Separation of Volatile Fatty Acids: Columns were prepared in glass tubes 30 cm. long by 19 mm. in diameter with a reservoir 15 cm. long by 33 mm. in diameter on one end and a drip tip on the other. The tubes were plugged with glass wool at the tip. Indicator solvent was prepared by dissolving 700 mg. of brom cresol green in 700 ml. of ethylene glycol and mixing with this 296 ml. of distilled water and 4 ml. of 1 N NH_4OH. This solvent was mixed with 12 g. of silicic acid (Mallinckrodts No. 2847) in a mortar until a slightly moist powder was obtained. The amount of solvent varied from 7 to 9 ml. for different batches of silicic acid. The rate of flow of the columns was largely regulated by the amount of indicator solvent used. This blue-green powder was slurried in hexane containing 1% n butanol by volume. The slurry was poured into the tube carefully to avoid inclusion of air in the column. The column was packed at a pressure of about 5 lbs. per square inch. The optimum velocity of flow of elution solvent was found to be 3 to 5 ml. per minute.

The samples of evaporated buffer prepared as described were dissolved in 2 ml. of 12% by volume H_2SO_4. Three g. of dry silicic acid was added and was mixed with the sample using a spatula until the mixture was powdery and homogeneous. Care was taken to see that evaporated material on the sides of the beaker was included. This powder was transferred quantitatively to glazed paper and thence to the column. Successive small portions of 1% butanol in hexane were allowed to run through the sample taking care that the column did not

run dry. The yellow bands of acid were observed as they developed on a green column. Threshold volumes of elution solvent for this method were approximately 20 ml. for C_6 and higher acids, 40 ml. for valeric, 85 ml. for butyric, 110 ml. for propionic and 210 ml. for acetic. After the butyric band had been eluted, the solvent was changed to 5% *n* butanol in hexane. Concentrations of over 15% *n* butanol were found to elute the indicator and so were not used. Volumes of each fraction were approximately 40 ml. for C_6 and higher acids, 45 ml. for valeric, 25 to 30 for butyric, 100 ml. for propionic and 90 ml. for acetic.

The samples were collected in 125 ml. Erlenmeyer flasks and 10 ml. of refluxed ethanol and 6 to 10 drops of thymol blue were added. The acids were stirred vigorously on a magnetic stirrer while carbon dioxide-free air was passed rapidly through the solution and were titrated with 0.1 N alcoholic KOH to a blue end point. It was noticed that the color change was fairly slow when only small amounts of acid were present. Unless base was added very slowly, the end point was exceeded before the color change occurred.

Three forages as substrates were fermented by rumen fluid from a cannulated sheep fed a mixed hay ration. The volatile fatty acid produced was estimated by steam distillation and by partition chromatography as described. Tables II and III give the results of the three determinations in duplicate which show that the technique gives quantitative and reproducible results.

TABLE II

YIELD OF ACIDS FROM THREE FERMENTATIONS IN DUPLICATE (ml. 0.1N KOH required to titrate acid)

	Sample 1		Sample 2		Sample 3	
	A	B	A	B	A	B
C_6 & higher	0.10	0.10	0.12	0.11	0.12	0.11
Valeric	0.20	0.19	0.20	0.19	0.20	0.21
Butyric	0.52	0.56	0.60	0.63	0.60	0.60
Propionic	1.56	1.56	1.70	1.70	1.52	1.54
Acetic	3.46	3.36	4.18	4.14	3.56	3.58
Total	5.84	5.77	6.80	6.77	6.00	6.04

TABLE III

YIELD OF VOLATILE FATTY ACID USING PARTITION CHROMATOGRAPHY AND STEAM DISTILLATION

Sample	(1) Chromatography Average ml. 0.1N KOH Used	(2) Steam Distillation Average ml. 0.1N KOH Used	$\frac{(1)}{(2)} \times 100$
1	5.81	5.80	100.2
2	6.79	6.82	99.6
3	6.02	6.04	99.7

Calculation of weight of acid was done as follows for each acid fraction:

Titre x normality x aliquot factor x molecular wt. of acid = wt. of acid

The C_6 and higher acid fraction was assumed primarily to be C_6 acid and so the molecular weight of the C_6 acid was used in calculations. In some experiments the valeric and C_6 acid fractions were collected together because of their extremely small quantities. In trials where these 2 fractions were collected separately, these acids

were roughly in the ratio of 2 equivalents of valeric acid to 1 of C_6 acid. The molecular weight, therefore, was calculated for the combined fraction as:

$$\frac{2 \times 102 \text{ (molecular wt. of valeric)} + 1 \times 116 \text{ (molecular wt. of } C_6\text{)}}{3}$$

$$= 106.7$$

Control: As will be observed later, the absolute values for volatile fatty acid production in Experiment 1 are relatively low compared to those in all other experiments. This is because the method used to determine a control value for the inoculum gave values that exceeded half of the total titrable fatty acid. Control values for Experiment 1 were obtained by determining the amount of fatty acid produced when the inoculum alone was fermented in the artificial rumen. The levels of acid obtained in this way represented not only that acid normally present in rumen fluid, but also the products of fermentations of dry matter suspended in the inoculum. It was assumed that freshly added substrate would be more likely to be attacked by the bacteria of the inoculum than would the fermentable material present in strained rumen fluid taken from sheep 3 to 4 hours after they were fed. For this reason, it was felt that correction should be on the basis only of acid added *per se* at the time of inoculation. This assumption is supported by the data of Hale *et al*. (1940, 1947) which indicated that practically all of the digestion of alfalfa hay *in vivo* occurred within the first 12 hours of fermentation. Although this assumption may not be entirely valid, it appeared to provide a relatively logical basis for control correction.

Control values in all experiments except Experiment 1 were determined by chromatographic measurement of the fatty acid present

in the buffer solution surrounding a fermentation sac of the artificial rumen which contained 10 ml. of inoculum and 3 to 4 drops of 40% formaldehyde and had been refrigerated for the period of incubation of the test fermentation.

This technique of separation and estimation of the volatile fatty acids was used routinely during the experiments reported.

EXPERIMENT 1

Object: To ascertain if differences can be observed in the production of volatile fatty acids, the proportions of volatile fatty acids produced or in the dry matter loss from the digestion sacs, when feeds of obviously different quality are fermented in the miniature artificial rumen by organisms from the rumina of sheep fed rations of low and high quality forage.

Description of Forages: Three forages were selected in an effort to obtain three hays of obviously varying quality. The high quality hay was carefully selected green, immature, leafy alfalfa. Good mixed grass-alfalfa hay of medium maturity was used as an intermediate sample and clean, bright oat straw as one of relatively low quality. The alfalfa was selected by hand from a large sample, while the mixed hay and straw were representative samples from several bales which were later fed to the cannulated sheep. The feeds were ground in a Wiley mill through a 40 mesh seive and were stored under refrigeration. The crude protein and crude fiber content of these feeds is given in Table IV.

TABLE IV

PARTIAL ANALYSIS OF THREE FORAGES FOR EXPERIMENT 1

Forage	Crude Protein %	Crude Fiber %
Alfalfa	18.0	20.4
Mixed Hay	12.5	26.2
Oat Straw	5.9	32.1

Experimental: The three forages described were fermented for 24 hours in the artificial rumen. Two rumen cannulated sheep were fed

the straw and two others the mixed hay selected for use as substrates. Pooled samples of inocula drawn from each pair of sheep were used to digest each of the three forages while a third series of artificial rumen sacs were inoculated with a mixture of equal parts of rumen fluid from both pairs of sheep. Hereinafter the three samples of rumen fluid obtained from the sheep on these specified rations will be referred to as hay inoculum, straw inoculum and combined inoculum.

Volatile fatty acids were separated and estimated and dry matter loss was determined. Two trials were conducted in duplicate.

Results and Discussion: Total fatty acid production and dry matter loss are given in Table V. Analyses of variance for these data are presented in Tables VI and VII.

It is apparent from these data that significantly different amounts of fatty acids are produced and significantly different proportions of dry matter are lost when feeds of obviously different quality are fermented or when inocula from sheep on different quality rations are used. A relatively close association appears to exist between the results obtained by fermentation assay and the crude fiber and crude protein content of the feeds. Feeding trials were not conducted with these forages so the digestibilities of the substrates were not available, but it seemed possible that a direct relationship between some measure of in vivo forage utilization and the data obtained by these techniques might exist.

Differences in the activity of inocula are apparently of approximately the same magnitude as the differences in the quality of the forages fed the animals used as source of inoculum.

TABLE V

VOLATILE FATTY ACID PRODUCTION AND DRY MATTER LOSS AS AFFECTED BY SUBSTRATES AND INOCULA -- Experiment 1

		(1) From Sheep Fed Hay		(2) From Sheep Fed Straw		(3) Mixture of (1) and (2)	
Substrates		Total Fatty Acids*	Dry Matter Loss %	Total Fatty Acids*	Dry Matter Loss %	Total Fatty Acids*	Dry Matter Loss %
Alfalfa	1)	107.4	62.9	82.9	55.2	95.0	56.4
	2)	111.4	68.5	79.0	52.3	94.2	57.7
Mixed Hay	1)	93.8	58.3	50.1	47.7	73.4	53.6
	2)	88.7	63.3	47.2	47.9	71.4	52.6
Straw	1)	69.5	47.5	40.1	40.6	55.3	45.9
	2)	72.1	45.9	42.6	42.0	63.4	42.7

*Expressed in mg. per 500 mg. substrate

TABLE VI

ANALYSIS OF VARIANCE -- VOLATILE FATTY ACID PRODUCTION
Experiment 1

Source of Variation	DF	MS	F
Between Inocula	2	1687.36	49.63**
Between Substrates	2	2204.06	64.83**
Interaction	4	34.00	4.13*
Error	9	8.24	

*Significant at $P < 0.05$
**Significant at $P < 0.01$

TABLE VII

ANALYSIS OF VARIANCE -- DRY MATTER LOSS
Experiment 1

Source of Variation	DF	MS	F
Between Inocula	2	156.36	34.21**
Between Substrates	2	337.45	73.84**
Interaction	4	9.96	2.18
Error	9	4.57	

**Significant at $P < 0.01$

Tables VIII, IX and X give the relative proportions of propionic and acetic acid with analyses of variance.

That there is a difference in the type as well as extent of fermentation by inocula from different sources is evident in the higher percentage of propionic acid produced from forages fermented by the straw inoculum. This suggests that the rumen population changes in numbers and in types of organisms according to the ration fed and that these changed populations effect different types of fermentations. High production of propionic acid seems to be associated not only with the low quality inoculum, but also with a lower quality of substrate. Although analysis of variance does not demonstrate a significant difference between substrates in the relative proportions of fatty acid produced it is evident from the significant mean square for interaction that substrates did have some effect on these proportions. A larger fraction of propionic acid seemed to be produced not only when the straw inoculum was used, but also when the lower quality feeds were fermented by that inoculum. The quality of substrate did not appear to affect the relative proportions of fatty acids produced by fermentations with the hay inoculum. It is possible that the higher protein or some other factors in the higher quality forages could have affected the population of the straw inoculum in such a way that the acetic acid producing ability was increased or that the capacity to produce propionic acid was depressed, while the hay inoculum had sufficient of these factors and was not affected by an excess of them.

The pooled inoculum produced, in all cases, results intermediate between those of the other two inocula. Although use of such

TABLE VIII

PERCENTAGES OF VOLATILE FATTY ACIDS PRODUCED
Experiment 1

		(1) From Sheep Fed Hay		(2) From Sheep Fed Straw		(3) Mixture of (1) and (2)	
Substrates		Acetic as % of Total	Propionic as % of Total	Acetic as % of Total	Propionic as % of Total	Acetic as % of Total	Propionic as % of Total
Alfalfa	1)	60.8	20.0	46.9	34.6	50.9	30.0
	2)	59.5	23.8	47.3	35.5	52.4	28.2
Mixed Hay	1)	62.5	21.5	45.9	37.1	53.4	28.4
	2)	63.2	20.6	41.2	40.8	52.6	27.4
Straw	1)	65.1	20.0	40.8	48.0	53.2	33.1
	2)	64.3	20.2	37.2	46.7	51.3	31.9

TABLE IX

ANALYSIS OF VARIANCE -- ACETIC ACID AS % OF TOTAL ACID
Experiment 1

Source of Variation	DF	MS	F
Between Inocula	2	547.86	25.95**
Between Substrates	2	1.78	.08
Interaction	4	21.10	8.48**
Error	9	2.49	

**Significant at $P < 0.01$

TABLE X

ANALYSIS OF VARIANCE -- PROPIONIC ACID AS % OF TOTAL ACID
Experiment 1

Source of Variation	DF	MS	F
Between Inocula	2	569.01	21.16**
Between Substrates	2	37.72	1.40
Interaction	4	26.89	13.09**
Error	9	2.05	

**Significant at $P < 0.01$

inoculum offered a source of control, it was discarded as a routine procedure in the interests of time. In subsequent experiments only the hay and straw inocula were used.

It was felt that methods employed in this experiment were adequate to detect differences between the fatty acid production and dry matter loss of different forages when fermented by inocula from different sources and that the experimental techniques gave sufficiently consistent results for routine assays.

On the basis of this experiment it was felt that there was justification in continuing the project to determine if a suitable method for the evaluation of forage quality might be devised using the miniature artificial rumen.

Summary:

1. A high, a medium and a low quality forage were fermented in the artificial rumen using for inocula, rumen fluid from sheep fed the medium and the low quality roughages that were used as substrates. The volatile fatty acids were separated and estimated and the dry matter loss determined.
2. A real difference was observed between substrates in the amount of volatile fatty acid produced and in the percentage of dry matter loss but not in the relative proportions of propionic and acetic acid produced.
3. A real difference was observed between inocula in the amount of volatile fatty acid produced, in the percentage of dry matter loss and in the relative proportions of propionic and acetic acid produced.
4. On the basis of the observed differences in volatile fatty acid production and dry matter loss which were roughly in

proportion to the assumed quality of the forages tested, it was decided to extend the investigation to feeds of known quality as determined by digestibility trials.

EXPERIMENT 2

Object: To determine what relationships may exist between forage quality ratings as determined by the artificial rumen technique and those determined by digestion trials.

Description of Forages: Eleven samples of forage were kindly supplied by Dr. W. J. Pigden, Central Experimental Farm, Ottawa. These were part of a group of 13 forages which had been used in digestion trials during the winter of 1950-51 by the Department of Animal Husbandry at the University of Saskatchewan. The results of these trials, together with the proximate composition of the forages, were published by Bell *et al*. (1952). The digestibility values used in the present investigation are those listed by Pigden (1955). Description of the forages, their proximate analysis and digestibility coefficients are given in Tables XI, XII and XIII. The samples were mixed thoroughly, ground in a Wiley mill through a 40 mesh sieve and stored under refrigeration.

Trial 1

Object: To determine for 11 forages the degree of association that might exist between digestibility coefficients and values for fatty acid produced and dry matter lost after incubating these forages for 24 hours in the artificial rumen.

Experimental: The 11 forages described were fermented for 24 hours in the miniature artificial rumen. Two series in triplicate were fermented, one using hay inoculum and one using straw inoculum. Production of volatile fatty acid and dry matter loss were determined as previously described.

Results: The data for weight of volatile fatty acid produced

TABLE XI

GENERAL DESCRIPTION OF FORAGES USED IN DIGESTIBILITY STUDIES

(Bell *et al*. 1952)
Experiment 2

Name	Harvest Date 1950	Description
Kochia, 1st stage (Kochia scoparia (1.) Schrad.)	July 7, 11	16-20" high, pre-bloom, fine stemmed, leafy. Cured artificially in 30 hours.
Alfalfa (Medicago sativa L.)	July 4	12-18" high, 50% in flower, appearance good, from test plots. Cured artificially for 24 hours.
Brome grass (Bromus inermis Leyss.)	July 7	24-30" high, in full flower, quite leafy, from test plots. Cured artificially for 16 hours.
Intermediate wheatgrass (Agropyron intermedium (Host) P.B.)	July 21	24-30" high, in full flower. Rather coarse in appearance. Cured artificially for 16 hours.
Russian wild rye (Elymus junceus Fisch.)	June 29	18-24" high, 50% in flower, appeared coarse and stemmy, from test plots, stored over night in barn loft in 10" layer, then dried artificially for 11 hours.
Oat Hay (Avena sativa L.)	Aug.9	36" high, in late dough stage, beginning to ripen. Cured artificially in 24 hours.
Canadian wild rye (Elymus canadensis L.)	July 22	20-24" high, in full flower, moderately leafy, stems coarse. Curing completed indoors in 24 hours without heat.
Brome grass straw (Bromus inermis leyss.)	Aug.7	From test plots for brome grass seed production. Excellent quality. Field cured.
Kochia, 2nd stage (Kochia scoparia (1.) Schrad.)	Aug. 1	36-40" high, stems up to 1/2" diameter, leafy, Cured artificially in 40 hours.
Kochia, 1st stage (Kochia childsii. Hort. ex. Mollers)	Aug. 18, 22	12-24" high in very thick stand. Cured artificially.
Marsh Hay (predominantly Carex spp.)	July 7	12-18" high. Grown at Dundurn Marsh. Field cured, baled. Fair quality. Somewhat over-mature.

TABLE XII

CHEMICAL COMPOSITION OF ELEVEN FORAGES (% OF DRY MATTER)*
Experiment 2

Forage	Crude Fiber	Crude Protein	Ash	Lignin
Kochia scoparia 1.	19.7	21.5	19.2	7.7
Alfalfa	25.7	19.8	8.9	8.7
Brome grass	29.4	13.4	7.1	9.5
Intermediate wheatgrass	34.3	9.7	6.4	10.4
Russian wild rye	22.3	13.4	7.2	11.4
Oat hay	29.5	10.7	8.0	9.4
Canadian wild rye	22.6	12.0	7.3	10.9
Brome grass straw	33.5	9.4	8.3	10.7
Kochia scoparia 2.	35.6	11.2	12.2	10.5
Kochia childsii	33.5	10.8	11.5	11.8
Marsh hay	33.5	6.8	6.2	13.7

TABLE XIII

DIGESTIBILITY COEFFICIENTS AND DIGESTIBLE PROTEIN OF ELEVEN FORAGES*
Experiment 2

Forage	Crude Protein	Dry Matter	Organic Matter	Digestible Protein
Kochia scoparia 1.	82.5	68.9	67.5	17.7
Alfalfa	80.9	65.4	68.1	16.0
Brome grass	68.7	63.6	65.9	9.2
Intermediate wheatgrass	65.3	61.3	61.7	6.3
Russian wild rye	73.7	60.4	62.2	9.9
Oat hay	68.9	59.4	60.7	7.4
Canadian wild rye	66.8	58.4	65.1	8.0
Brome grass straw	57.5	57.2	58.7	5.4
Kochia scoparia 2.	69.7	55.8	53.3	7.8
Kochia childsii	64.1	55.4	51.5	6.9
Marsh hay	36.5	53.9	56.5	2.5

* Pigden (1955)

and percent dry matter loss are presented in Table XIV. The analyses of variance for these results are presented in Tables XV and XVI.

TABLE XIV

THE AVERAGE FATTY ACID PRODUCTION AND DRY MATTER LOSS FROM ELEVEN FORAGES OF KNOWN DIGESTIBILITY -- Experiment 2, Trial 1

	Fatty Acid Production*		Dry Matter Loss %	
Forage	Hay Inoculum	Straw Inoculum	Hay Inoculum	Straw Inoculum
Kochia scoparia 1.	197.9	147.0	64.6	55.5
Alfalfa	217.1	143.5	59.8	49.7
Brome Grass	181.3	138.2	50.3	40.8
Intermediate wheat grass	184.1	132.1	46.4	34.0
Russian wild rye	166.6	120.8	42.8	32.2
Oat hay	176.7	126.9	43.2	33.2
Canadian wild rye	185.7	134.9	49.4	37.5
Brome straw	177.2	129.2	42.5	34.0
Kochia scoparia 2.	185.1	140.2	53.7	44.9
Kochia childsii	180.7	126.9	49.3	37.3
Marsh hay	167.3	114.2	39.5	24.4

*Expressed as mg. per 500 mg. of substrate

TABLE XV

ANALYSIS OF VARIANCE FOR FATTY ACID PRODUCTION
Experiment 2, Trial 1

Source of Variation	DF	MS	F
Between Inocula	1	43,684.9	440.8**
Between Substrates	10	791.0	8.0**
Interaction	10	99.1	11.9**
Error	44	8.3	

**Significant at $P < 0.01$

TABLE XVI

ANALYSIS OF VARIANCE FOR DRY MATTER LOSS
Experiment 2
Trial 1

Source of Variation	DF	MS
Between Inocula	1	1,909.04**
Between Substrates	10	402.9**
Interaction	10	5.98
Error + Triplicates	44	3.40

**Significant at $P < 0.01$

The standard deviation associated with variation between triplicates for fatty acid was 1.6 mg., while that for dry matter loss was 1.8%. This relatively low variation when compared with the differences between forages provides additional evidence that the methods used are reproducible and can detect necessary differences.

The figures for acid production and dry matter loss indicate that forages of different quality are fermented to a significantly different extent in the artificial rumen and that inocula from different sources vary significantly in their fermenting ability.

Coefficients of correlation were calculated to see if the variations in the fermentation of forages in the artificial rumen were associated with variations in the quality of the substrates as determined in feeding trials. These coefficients, summarized in Table XVII, show that association between the results of in vitro fermentations and the results of feeding trials does exist.

It is of interest to note that the measurements of fatty acid production and dry matter loss are more closely correlated with crude and digestible protein than with percent dry matter digestibility. This association offers added evidence for the importance of the protein fraction in the utilization of forages.

TABLE XVII

COEFFICIENTS OF CORRELATION BETWEEN RESULTS OF IN VITRO FERMENTATION AND RESULTS OF FEEDING TRIALS -- Experiment 2

	Fatty Acid Production		Dry Matter Loss	
	Hay Inoculum	Straw Inoculum	Hay Inoculum	Straw Inoculum
Dry Matter Digestibility	.647*	.708*	.718*	.730*
Crude Protein	.775**	.768**	.882**	.884**
Digestible Protein	.772**	.771**	.888**	.912**

*Significant at $P < 0.05$
**Significant at $P < 0.01$

While the results with the artificial rumen are significantly correlated with the dry matter digestibility coefficients of the forages tested, the value of this method in assessing or predicting *in vivo* results has not been established. Several limitations are still apparent. The number of forages is quite limited and these feeds were grown in one area. While the roughages include several different species, several important forage crops are not included, and also 3 of the 11 samples were *Kochia* species which are not yet widely used as forage crops nor definitely established as valuable roughages.

The magnitude of the coefficients of correlation indicates that only approximately 40 to 50% of the variation in results obtained by the artificial rumen method is associated with the variation in the determined digestibility of dry matter. Prediction on the basis of a 50% association would be open to considerable question, especially when the association is based on so few samples.

For 9 of the forages the association between *in vitro* and *in vivo* measurements appears to be quite high, but for 2 samples, *Kochia scoparia* 2., and *Kochia childsii* this association is very low. This

low association is probably responsible in a large part for the comparatively low coefficients of correlation. This anomalous behavior could be due to several factors. It was felt, therefore, that some investigation should be made in order to see if this difficulty could be overcome or if its cause could be discovered.

Summary:

1. Eleven feeds of known digestibility were fermented for 24 hours in the miniature artificial rumen using the hay and the straw inocula described in Experiment 1. The volatile fatty acids were separated and estimated and the dry matter loss was determined.
2. Real differences were detected between forages and between inocula with regard to volatile fatty acid production and dry matter loss.
3. Coefficients of correlation between the volatile fatty acid production and dry matter loss and dry matter digestibility were significant. Fatty acid yield and dry matter loss were highly significantly correlated with crude and digestible protein.
4. The value of the demonstrated correlation between *in vitro* and *in vivo* results in prediction of forage quality was not considered adequately established.
5. It was decided to extend the investigation to attempt to obtain a higher degree of association between the artificial rumen measurements and the results of feeding trials with special emphasis on the behavior of two samples of *Kochia*.

Trial 2

Object: To determine the effect of time of digestion and washed cell inocula on the predictability of forage quality by the miniature artificial rumen technique.

Experimental: Series 1 involved the fermentation of 4 sets of samples of the 11 forages. Two sets were fermented by the hay inoculum, one for 18 hours, the other for 48 hours. The remaining 2 sets were fermented by the straw inoculum for 18 and 48 hours respectively. The loss of dry matter was determined.

For Series 2, washed cell suspensions were prepared from the hay and the straw inocula using the method previously described. These inocula will hereinafter be referred to as washed hay and washed straw inocula.

The washed hay inoculum was used to ferment 2 sets of samples of the 11 forages, one for 24 hours and the other for 48 hours. The washed straw inoculum was used to ferment 2 more sets of the 11 forages, one for 24 hours, the other for 48 hours. The dry matter loss was determined for all 4 groups.

Results: The dry matter loss data for the Series 1 are given in Table XVIII. Coefficients of correlation with the dry matter digestibility coefficients of the 11 forages are given in Table XIX. The r values for dry matter loss from Table XVII are included as values for a 24 hour fermentation for comparison.

These data indicate that the association between dry matter loss as measured at the 18th hour of *in vitro* fermentation and the digestibility coefficients for dry matter as determined in feeding trials is lower than is the association at the 24th or 48th hour of incubation. The results of the 48 hour fermentation seem to be almost as closely

correlated with the dry matter digestibility data as those for the 24 hour period when the hay inoculum is used. There appears to be a much greater association between *in vitro* and *in vivo* digestion when the feeds are fermented for 48 hours using the straw inoculum than there is for the 24 hour period of incubation.

TABLE XVIII

AVERAGE PERCENT DRY MATTER LOSS
Series 1, Experiment 2

Forage	Hay Inoculum		Straw Inoculum	
	18 hrs.	48 hrs.	18 hrs.	48 hrs.
	%	%	%	%
Kochia scoparia 1.	52.6	71.0	48.1	68.1
Alfalfa	47.0	69.1	45.0	60.8
Brome Grass	36.0	59.2	32.8	55.3
Int. wheat grass	34.7	62.3	31.9	53.8
Russian wild rye	28.6	57.6	25.8	53.2
Oat hay	33.4	60.2	32.7	48.4'
Canadian wild rye	36.5	60.0	27.8	52.3
Brome straw	32.6	59.4	27.3	52.8
Kochia scoparia 2.	48.3	63.3	41.3	52.3
Kochia childsii	44.2	62.2	31.7	50.6
Marsh hay	29.0	59.1	21.3	40.8

The dry matter loss data for fermentations using washed cell inocula are presented in Table XX. Coefficients of correlation with the dry matter digestibility coefficients of the 11 forages tested are given in Table XXI.

TABLE XIX

COEFFICIENTS OF CORRELATION OF DRY MATTER LOSS AS MEASURED IN THE ARTIFICIAL RUMEN WITH THE DRY MATTER DIGESTIBILITY COEFFICIENTS OF ELEVEN FORAGES

Series 1, Experiment 2

	Hay Inoculum			Straw Inoculum		
	18 hrs.	24 hrs.	48 hrs.	18 hrs.	24 hrs.	48 hrs.
r	.444	.718*	.663*	.685*	.730*	.889**

* Significant at $P<0.05$

** Significant at $P<0.01$

TABLE XX

PERCENT DRY MATTER LOSS USING WASHED CELL INOCULA

Series 2, Experiment 2

	Washed Hay		Washed Straw	
Forage	24 hrs.	48 hrs.	24 hrs.	48 hrs.
	%	%	%	%
Kochia scoparia 1.	46.8	53.9	48.4	55.6
Alfalfa	43.8	57.7	43.4	55.0
Brome	31.8	41.6	33.0	48.9
Int. wheat grass	26.8	30.6	31.2	36.6
Russian wild rye	24.8	36.2	29.2	42.6
Oat hay	25.8	36.4	30.6	40.1
Canadian wild rye	24.0	40.6	25.8	38.8
Brome straw	24.6	38.4	25.6	38.2
Kochia scoparia 2.	30.8	47.4	39.9	49.4
Kochia childsii	26.5	42.8	30.6	40.8
Marsh hay	22.6	25.4	20.9	28.9

TABLE XXI

COEFFICIENTS OF CORRELATION OF DRY MATTER LOSS USING WASHED CELL INOCULA WITH DIGESTIBILITY COEFFICIENTS OF DRY MATTER

Series 2, Experiment 2

	Washed Hay		Washed Straw	
	24 hrs.	48 hrs.	24 hrs.	48 hrs.
r	.829**	.606	.741**	.748**

** Significant at $P<0.01$

The use of washed cell inocula increased the accuracy of the miniature artificial rumen method in the prediction of forage quality only slightly. The level of significance was raised to $P<0.01$ in 3 out of 4 trials using washed inocula, while only one of the 4 trials using rumen fluid was significant at this level. However, the degree of association between *in vitro* and *in vivo* measurements was still not adequate for reliable prediction of forage quality.

Summary:

1. The effects of time of incubation and of washed cell inocula on the correlation between dry matter loss in the miniature artificial rumen and dry matter digestibility coefficients of 11 forages were studied.
2. Measurement of dry matter loss after 18 hours of incubation decreased the association between *in vitro* and *in vivo* data.
3. Results for dry matter loss after 48 hours of incubation were more closely correlated with *in vivo* results when the straw inoculum was used, but measurements when the hay inoculum was used showed no such trend.
4. The dry matter loss when washed cell inocula were used was slightly more closely correlated with coefficients of dry matter digestion than when rumen fluid was employed as inoculum.
5. While a significant degree of correlation was observed in all assays, it was felt that the magnitude of association was still not adequate to predict the relative digestibility of forages *in vivo* using these *in vitro* results.

General Discussion

In Trial 2 some possibilities of discovering the cause of or a correction for the uncertain predictability noticed in Trial 1 were investigated. Pigden (1956) noticed that forages of high quality tended to initiate fermentation more quickly than did those of lower quality. Because of this it was thought that perhaps measurements taken after a shorter period of fermentation might improve estimates of forage quality by detecting this rapid initiation of digestion. However, the fermentations after 18 hours of incubation did not appear to follow Pigden's observation sufficiently well to be used as an index of quality of roughage. While some of the forages of high quality did begin decomposition very early and some of the lower quality hays took longer to begin fermentation, there were some low quality forages, especially *Kochia scoparia* 2.and *Kochia childsii*, which began fermentation as rapidly as many forages of higher quality. This can be observed in Figure 1.

Measurement of dry matter loss after 18 hours of incubation in these experiments tended to decrease the usefulness of the measurements in predicting forage quality. Fermentations which lasted less than 18 hours did not seem reliable. One trial using an incubation time of 12 hours was abandoned because results were extremely variable and duplication of results was not successful.

The primary criterion of forage quality used in comparing the *in vitro* results with the data from feeding trials was the digestibility coefficient of dry matter. It was assumed that this coefficient represented a maximum *in vivo* fermentation of an individual forage, and that this maximum was reached only after a time longer than 24 hours. The 48 hour fermentation was used to ascertain if this longer fermentation would give a better approximation of *in vivo* digestion than

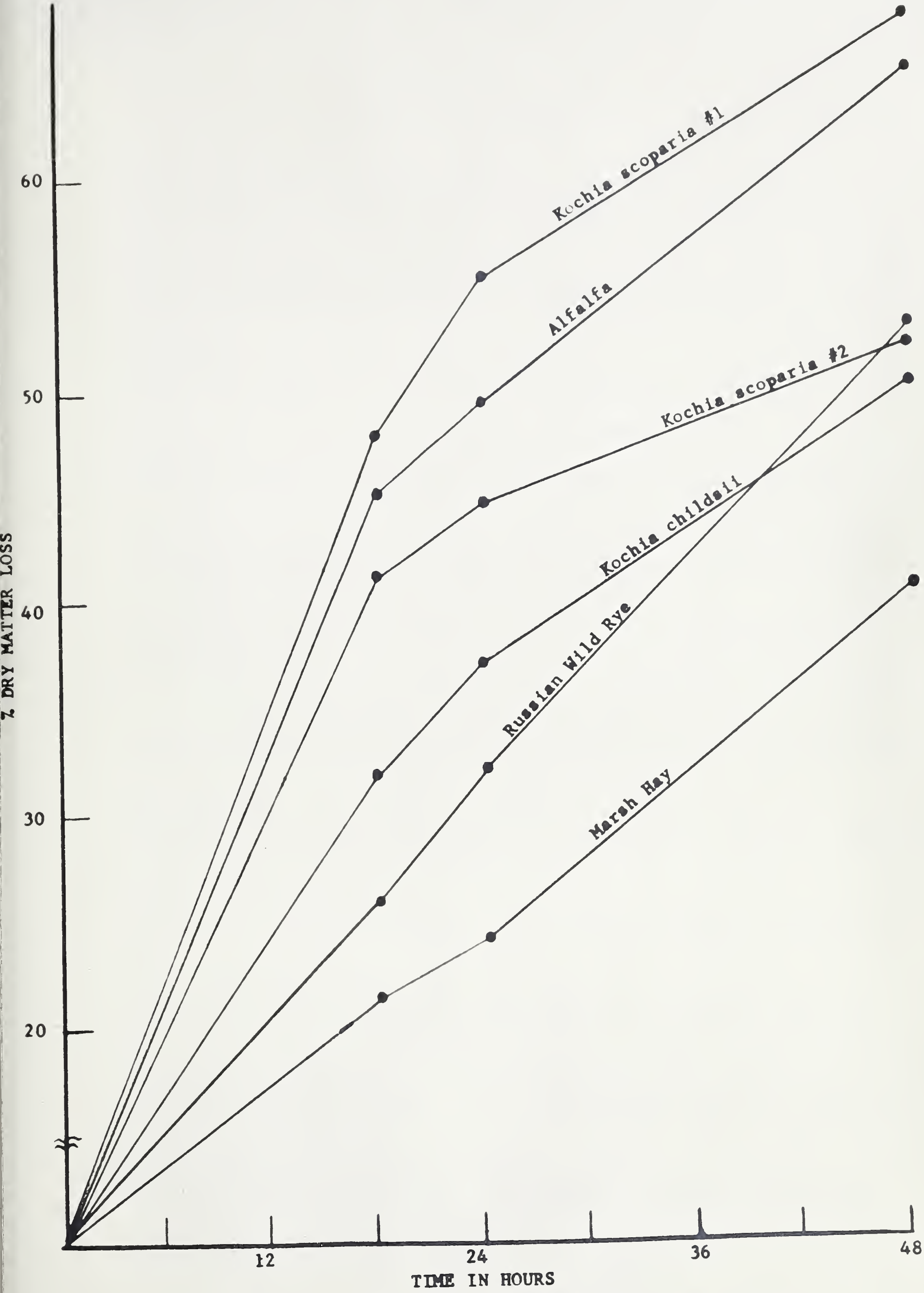


FIGURE 1. Digestion of Forages vs. Time Using Straw Inoculum

would one which lasted only 24 hours. There was some indication using the straw inoculum that this might be so. The fermentation trials with the hay inoculum, however, tend to indicate that the predictability of digestibility from results at 48 hours was less than from those at 24 hours, although it is doubtful if the difference between the two coefficients of correlation, .718 and .683, can be considered as indicating a real difference in association, especially when so few samples are involved. The difference in association involving the dry matter loss produced by different inocula might suggest that there is an optimum time for each individual inoculum at which time the greatest association can be obtained between _in vitro_ and _in vivo_ fermentations. Because sacs tend to disintegrate in fermentations longer than 48 hours, digestions could not be carried out beyond this time.

It is generally accepted that rumen fermentation is accelerated if small amounts of readily available energy or available nitrogen are added. In addition to this, it has been shown that there are factors in whole rumen fluid which promote the utilization of cellulose. These factors include volatile fatty acids and amino acids. When whole rumen fluid is used as inoculum, considerable amounts of energy, protein, fatty acids, amino acids and other factors are added to the fermentation mixture. These materials might very possibly confuse the pattern of fermentation of various forages by supplying otherwise deficient nutrients. Forages of low quality may ferment more thoroughly when supplied with available energy or nitrogen from rumen fluid than would ordinarily be possible. For example, Burroughs and Gerlaugh (1949c) found that added soybean oil meal greatly

increased the dry matter digestibility coefficient of corn cobs. In an effort to increase the predictability of dry matter digestibility using the miniature rumen, and to avoid the introduction of these confusing factors into the fermentation, washed cell inocula were used.

The data in Table XX show that the differences between fermentations using washed hay and washed straw inocula were not so great as were those between fermentations when the inocula were not washed. The straw inoculum appears to be even slightly more active than the hay inoculum. This suggests that the differences between inocula may be dependent to a very great extent on the nature and amount of dry matter suspended in the rumen fluid. Differences in bacterial populations must certainly exist but these differences apparently have less effect on forage digestion than do the extraneous materials in rumen fluid.

It was observed that a considerable amount of the variation from expected results was due to the excessively high values for Kochia scoparia 2. and Kochia childsii. In all cases these two feeds produced higher results than could be expected on the basis of their proximate analyses or the results of feeding trials. Several factors may have influenced fermentations with these forages.

1. The Kochia samples are extremely high in ash content as shown in Table XII. Moreover, this ash has a relatively different composition from that of most forages in that the silica content of Kochia is about 1.6% compared to 2.3 to 4.1% for most grass hays, Bell et al. (1954). This low silica ash was probably more soluble than other ashes high in this element. A greater loss in weight

may have occurred during fermentation and dialysis because of a loss of soluble minerals.

It was thought that a correction might be made in order to minimize the effects of the high, relatively soluble ash content of the two Kochia samples. However, a correction of the values for dry matter loss based on ash content cannot be valid in the light of the data for volatile fatty acid production. To make such a correction it is necessary to assume that there is a greater loss of ash from the Kochia species which makes the apparent fermentation of organic matter seem to be more than it actually is. But on this basis, the production of fatty acid from the organic matter of these forages would be even greater than is apparent from the high levels of acids from these species even before such correction. If these two species produce more volatile fatty acid from less organic matter than do feeds of higher digestibility and lower ash content, corrections on the basis of ash content cannot be valid. It would therefore, be a questionable practice to correct the dry matter loss data because the fatty acid figures indicate that the two Kochia samples are actually fermented more thoroughly than would be expected on the basis of their dry matter digestibility coefficients.

2. There may be factors in the Kochia species which accelerate cellulolytic activity. The nitrogen may be in a more available form and the ash may contain some stimulatory factors for cellulose digestion.

3. The main stems of mature Kochia plants are coarse and woody with a pithy center. The forages were only very coarsely ground in the feeding trials, Bell *et al*. (1952), while the samples

used in artificial rumen studies were finely ground. There is a possibility that the physical condition, the high ash content or some other property of these *Kochia* samples may have reduced digestibility *in vivo* but not *in vitro*.

4. A sampling error could easily have occurred because the ground *Kochia* samples were very heterogeneous and were sampled and subsampled several times before these trials. The possibility that the samples of *Kochia* used in present trials were not representative is suggested by the fact that Pigden (1955) found that neither *Kochia scoparia* 2. and *Kochia childsii* gave abnormally high fermentations as measured by the disappearance of anthrone carbohydrate.

Although many hypotheses may be advanced to explain the anomalous behavior of *Kochia scoparia* 2. and *Kochia childsii*, none of these theories can be proven without extensive investigation into the properties of these species. This is beyond the scope of the present experiment.

The possible use of the miniature artificial rumen method in the evaluation of most common forages might be considered by excluding the two mature *Kochia* samples which present many problems perhaps unrelated to ultimate feeding value. If a sampling error or some other change has occurred, the elimination of these two samples is valid. The fact that these species are not yet widely used as forages might also provide some justification for deleting results for these samples.

Simple correlation was calculated between results obtained *in vitro* for 9 forages excluding 2 samples of *Kochia* and the dry matter digestibility coefficients of these 9 samples. These *r* values are given in Table XXII.

TABLE XXII

SIMPLE COEFFICIENTS OF CORRELATION OF DRY MATTER LOSS AND FATTY ACID PRODUCTION WITH DRY MATTER DIGESTIBILITY *IN VIVO*

Experiment 2

		11 Forages Including Kochias	9 Forages Excluding Kochias
Dry Matter Loss		r	r
Inoculum	Time		
Unwashed hay	24 hrs.	.718*	.908**
Unwashed hay	48 hrs.	.663*	.793**
Unwashed straw	24 hrs.	.730*	.931**
Unwashed straw	48 hrs.	.889**	.940**
Washed hay	24 hrs.	.829**	.908**
Washed hay	48 hrs.	.606	.824**
Washed straw	24 hrs.	.741*	.965**
Washed straw	48 hrs.	.748*	.938**
Fatty Acid Production			
Hay Inoculum	24 hrs.	.647*	.721*
Straw Inoculum	24 hrs.	.708*	.870**

*Significant at $P<0.05$
**Significant at $P<0.01$

These figures show a high degree of association. Predictability based on these levels of correlation would be sufficiently high to give reasonably accurate estimates for digestibility of dry matter.

Regression equations were calculated giving the regression of digestibility coefficient of dry matter on the dry matter loss for these 9 feeds. Regression coefficients (byx) and standard errors of estimate (Se) are given in Table XXIII for different inocula and fermentation periods.

TABLE XXIII

REGRESSION COEFFICIENTS AND STANDARD ERRORS OF ESTIMATE OF DRY MATTER DIGESTIBILITY ON DRY MATTER LOSS IN THE ARTIFICIAL RUMEN
Experiment 2

Dry Matter Loss		11 Forages Including Kochias		9 Forages Excluding Kochias	
Inoculum	Time	byx	Se	byx	Se
Unwashed hay	24 hrs.	.43	3.34	.48	2.12
Unwashed hay	48 hrs.	.71	3.62	.75	2.96
Unwashed straw	24 hrs.	.38	3.32	.44	1.79
Unwashed straw	48 hrs.	.59	2.27	.56	1.67
Washed hay	24 hrs.	.47	2.65	.45	2.10
Washed hay	48 hrs.	.29	3.87	.36	2.77
Washed straw	24 hrs.	.41	3.28	.50	1.19
Washed straw	48 hrs.	.42	3.19	.48	1.65

Analysis of covariance indicated no significant difference between the regression coefficients for each series of fermentations so the data were pooled and an overall regression coefficient calculated for 11 forages and for 9 forages.

For 11 forages, byx = .42, Se = 3.19 ($P<0.05$)

For 9 forages, byx = .47, Se = 2.03 ($P<0.01$)

The regression equations would then be:

For 11 forages $Y = a + .42\ X$

For 9 forages $Y = a + .47\ X$

where a represents a constant which is dependent upon the relative activity of the inoculum used. This constant might be determined for each inoculum used by digesting a few reference feeds

of known digestibility along with the forages being assayed.

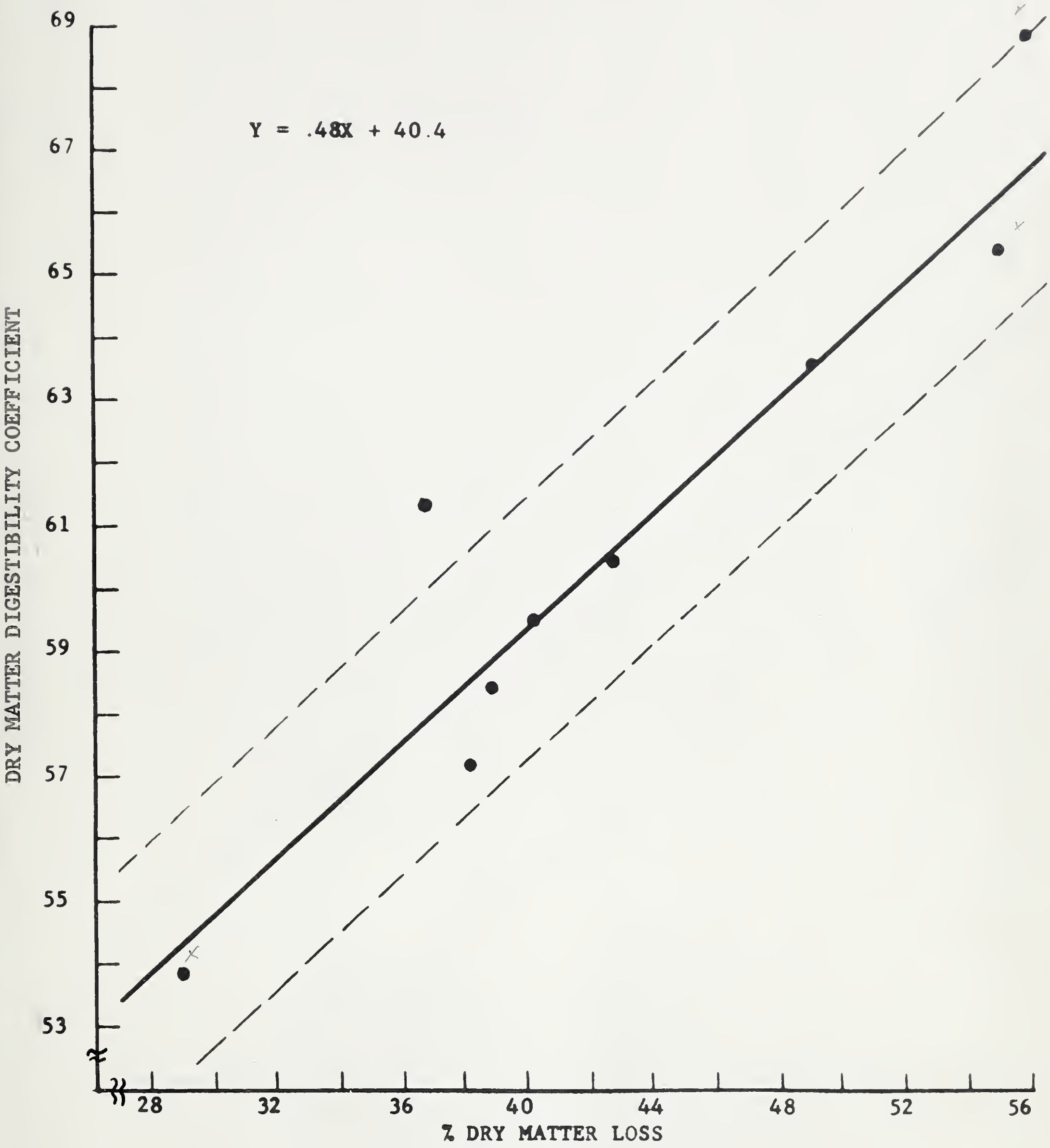
Typical regression lines for 9 and 11 forages are shown in Figures 2 and 3. These are the data for dry matter loss in a 48 hour fermentation with washed straw inoculum.

It is apparent that the predictability of the digestibility of feeds using the artificial rumen can be shown to be higher by the elimination of the 2 samples of *Kochia*. Whether or not such an exclusion is valid is a question which can be settled only on the basis of more extensive research. There are indications, however, that the techniques employed to determine the quality of forages are relatively reliable for most commonly used forage crops.

General Summary:

1. The techniques employed in the assessment of the quality of 11 forages gave results which showed significant correlations with forage quality as measured by digestibility trial results, but were not closely enough associated to be used as a basis for prediction of that quality.
2. The anomalous results from *Kochia scoparia* 2. and *Kochia childsii* were a source of considerable variation. Explanation of these results and possible corrections were considered to be inconclusive without further extensive research.
3. Associations on the basis of 9 feeds, excluding the 2 mature *Kochia* samples were sufficiently high to be used as a basis for prediction of forage quality.

FIGURE 2 Regression of Dry Matter Digestibility Coefficients on Dry Matter Loss *in vitro* for 9 Forages



EXPERIMENT 3

Object: To determine if results similar to those of Experiment 2 could be obtained using another set of forages.

Description of Forages: Six forages supplied by Dr. W. J. Pigden, Central Experimental Farm, Ottawa, were used. A description of these forages is given in Tables XXIV and XXV. These samples were ground in a Wiley mill through a 40 mesh sieve and stored under refrigeration.

Experimental: The feeds were fermented in the miniature artificial rumen and the dry matter loss determined. The inoculum used was a washed cell suspension of rumen fluid collected from 3 animals selected at random from 5 cannulated sheep fed on a ration of good mixed grass-alfalfa hay. This will hereinafter be referred to as a random inoculum.

Results and Discussion: The dry matter losses at 24 and 48 hours are given in Table XXVI. The simple coefficients of correlation with dry matter digestibility are .938 for the 24 hour fermentation and .818 for the 48 hour incubation period. These coefficients of correlation are significant at $P<0.01$ and $P<0.05$ respectively at 4 degrees of freedom.

While the number of samples is very small this association between *in vivo* and *in vitro* results appears to be adequate for assessment of forage quality by the artificial rumen technique. This supports the view expressed in Experiment 2 that the described techniques were satisfactory for most commonly fed forages. It also indicates that the nutritive value of mixed as well as botanically pure samples of forage can be estimated by this *in vitro* technique.

TABLE XXIV

DESCRIPTION OF FORAGES FED AT OTTAWA IN DIGESTIBILITY TRIALS AND RETAINED FOR ARTIFICIAL RUMEN STUDIES

Experiment 3

Forage	Description	1956 Date Harvested
Farmer's Mixture*	Predominantly timothy and brome grass with some alfalfa, medium maturity	July 27
Brome Grass	Growth still quite leafy, fairly mature	Sept. 18
Farmer's Mixture*	60% grasses, 40% legume, medium maturity	Aug. 9
Alfalfa-Timothy	Predominantly alfalfa, mature	Aug. 15
Farmer's Mixture*	50% grass, 50% legume, very mature	Aug. 16
Alfalfa-Timothy	Predominantly alfalfa, second cut 8" - 14" high, immature	Aug. 8

*A mixture of Timothy, Brome, Alfalfa, Red Clover and Ladino which changed markedly in species composition during the season with successive cuttings.

TABLE XXV

CHEMICAL COMPOSITION AND DIGESTIBILITY OF FORAGES FED AT OTTAWA IN DIGESTIBILITY TRIALS AND RETAINED FOR ARTIFICIAL RUMEN STUDIES

Experiment 3

Mixture	Ash	Crude Protein	Crude Fiber	Dry Matter Dig. Coef.
	%	%	%	%
Farmer's Mixture July 27, 1956	8.7	20.1	27.2	76.8
Brome Grass September 18, 1956	13.5	13.3	24.1	57.6
Farmer's Mixture August 9, 1956	8.5	19.4	23.1	71.7
Alfalfa-Timothy August 15, 1956	6.6	18.4	20.1	67.1
Farmer's Mixture August 16, 1956	6.9	15.9	27.9	62.3
Alfalfa-Timothy August 8, 1956	7.2	20.9	22.8	73.5

TABLE XXVI

DRY MATTER LOSS FOR SIX FORAGES
Experiment 3

Forage	24 hrs.	48 hrs.
	%	%
Farmer's Mixture July 27, 1956	37.4	57.3
Brome Grass September 18, 1956	30.8	51.2
Farmer's Mixture August 9, 1956	37.7	55.9
Alfalfa-Timothy August 15, 1956	35.1	51.5
Farmer's Mixture August 16, 1956	32.5	49.6
Alfalfa-Timothy August 8, 1956	39.4	53.0

The artificial rumen technique could be applied on a large number of samples taken at frequent intervals during crop maturation in order to determine the pattern of change in forage quality during this time.

Summary:

1. Six forages were fermented in the miniature artificial rumen by a washed random inoculum and the dry matter loss was determined.
2. The dry matter loss was significantly associated with the dry matter digestibility coefficients of the forages.
3. This assay supports the results of Experiment 2 using a different series of forages. Additional uses of the artificial rumen determination are suggested.

EXPERIMENT 4

Introduction: Methods of estimating forage quality involving proximate analysis have been shown to have several weaknesses. The assumptions used in calculation of total digestible nutrients have not been confirmed by actual feeding performance. Use of these assumptions in calculating the energy provided by a forage seems tedious and arbitrary when direct energy measurements can be made. For this reason workers are beginning to use the digestible energy relationships of feeds as indices of their value. Swift (1957) gave this summary of the relative advantages of digestible energy as a measure of nutritive value.

> "The measure of nutritive value as net energy, ideal from a theoretical standpoint, is sensitive to many factors including those quite apart from the nutritive composition of the feed. Also the unavoidable magnification of experimental error, even though kept to a minimum by elaborate and rigid control of experimental conditions, rules it out as a practicable procedure.
>
> "Metabolizable energy was considered to be a very satisfactory measure, but since its experimental determination requires that the energy of the urine and methane be determined, it was not adopted as a routine procedure available to the various stations.
>
> "Digestible energy, derived from direct determinations of the energy content of feed and feces was considered quite satisfactory from the standpoint of ease, speed, accuracy and reproducibility of results in the hands of various investigators. Like TDN it represents a feed-feces difference but is obviously more direct and accurate and free from empirical procedure and assumptions.
>
> "The very high correlation found between digestible energy and metabolizable energy lends further support for the use of digestible energy as a simple and meaningful routine measure of nutritive value."

Digestible energy is perhaps at present the most practicable measurement of the useful portion of a particular feed but there is an obvious difference between the proportion of feed that is useful

and the proportion of the energy requirement of an animal that is supplied by voluntary ingestion of feed. One forage may supply 2.5 Cal. per g. of feed while another supplies 2.0 Cal. per g. yet the first feed may be adequate for production while the second is not sufficient for maintenance. The difference lies not in the digestible energy supplied per unit of feed, but rather in the total energy supplied to the animal. Thus consumption rather than composition may well be the main factor in determining the absolute value of a feed.

Under conditions of *ad libitum* feeding, total consumption of roughage may depend as much or more on the speed at which the digestible portion is digested as on the proportion of undigestible material in the feed. Legumes, especially when mature, are higher in lignin and other undigestible nutrients than are grass hays yet they are frequently consumed more readily than the grass hays. Protein content is related to forage consumption although perhaps not directly. The level of protein affects the activity of the rumen microorganisms and in that way may increase speed of digestion. Moreover the more rapidly digestible parts of a plant, such as the leaves, are usually higher in protein.

Crampton (1957) has proposed that the voluntary consumption of a forage over an extended period may serve as a good practical index of the quality of that forage. He suggests that the level of intake is largely influenced by the rate of cellulose digestion. Because it is possible to approximate the rate of the digestion of the holocellulose fraction of forages in the miniature artificial rumen, it was proposed to ascertain if associations between results obtained by artificial rumen techniques and voluntary feed consumption

by ruminants could be demonstrated.

Description of Forages: Forages were kindly supplied by Dr. E. W. Crampton, Macdonald College. Two series of samples were assayed. Series 1 consisted of 5 samples which, at Macdonald College, had been cut at full bloom, cured under practical farm conditions and chopped when dry. Each of the 5 forages had been fed to 5 two-year old Cheviot ewes on a rotational basis for a period of eight weeks and voluntary feed consumption recorded. Data on the partial analysis of these feeds and their voluntary intake by the sheep as supplied from Macdonald College, are given in Table XXVII.

Series 2 consisted of another set of 5 forages from Dr. Crampton which had been chopped green into a wagon box in the field, dried in the box artificially and bagged. These had been fed to 5 Cheviot ewes on the basis of a randomized Latin Square. Voluntary feed consumptions were recorded over a three-week period. The crude protein content of these feeds and the data for voluntary intake as supplied from Macdonald College are given in Table XXX.

All forages were received in a finely ground condition and were stored under refrigeration.

Experimental: Both series of feeds were fermented in artificial rumen sacs using a washed random inoculum as described in Experiment 3. Two replicates in duplicate of Series 1 and duplicate samples of Series 2 were assayed. Volatile fatty acid production and loss of dry matter were determined.

Results:

Series 1 -- The dry matter loss and volatile fatty acid production data are given in Table XXVIII. Coefficients of correlation between these *in vitro* results and the voluntary feed consumption

TABLE XXVII

ANALYSIS OF FORAGES USED IN SERIES 1 Experiment 4

Forage Species	Crude Protein Content	Crude Fiber Content	TDN	Voluntary Dry Matter Intake	Gain in Body Weight
	%	%	%	g/day	lb.
Trefoil	15	34	59	1160	19
Red Clover	16	26	58	1070	13
Brome Grass	9	35	58	650	1
Timothy	6	36	56	490	-3
Oat Straw	2	42	52	260	-15

TABLE XXVIII

DRY MATTER LOSS AND FATTY ACID PRODUCTION FOR FORAGES IN SERIES 1 -- Experiment 4

Forage Species	Percent Dry Matter Loss		Fatty Acid Production mg. per 500 mg. forage	
	24 hrs.	48 hrs.	24 hrs.	48 hrs.
Trefoil	33.6	45.0	68.8	118.0
Red Clover	35.8	49.3	65.2	108.7
Brome Grass	20.7	34.8	41.8	82.0
Timothy	20.2	32.2	41.2	71.7
Straw	14.3	22.2	31.3	52.1

TABLE XXIX

SIMPLE CORRELATION OF DRY MATTER LOSS AND FATTY ACID PRODUCTION WITH FEED CONSUMPTION IN SERIES 1 -- Experiment 4

	Percent Dry Matter Loss		Fatty Acid Production	
	24 hrs.	48 hrs.	24 hrs.	48 hrs.
r	.973**	.957*	.986**	.996**

**Significant at $P<0.01$

*Significant at $P<0.05$

of these forages are given in Table XXIX. Inspection of these data reveals several interesting points.

1. Although the number of samples is low, the extremely high _r_ values indicate a very close association between voluntary feed consumption and the _in vitro_ fermentation as measured by either dry matter loss or fatty acid production.

2. Trefoil, which ranked first in voluntary consumption, ranked second in dry matter loss but gave the highest yeild of fatty acid of any of the forages.

3. Red clover, which ranked second in voluntary consumption, ranked first in dry matter loss, but produced less fatty acid than did trefoil.

4. Brome, timothy and oat straw ranked third, fourth and fifth respectively in voluntary consumption as well as in dry matter loss and fatty acid production.

No difference between replicates was demonstrated by an analysis of variance. This suggests that there was no measurable difference in the activity of the inocula employed even though they were taken from different sheep at different times. This is not always so, but, as has been stated, the use of a washed cell technique greatly reduces variation between inocula.

Series 2 -- The data for dry matter loss and fatty acid production are given in Table XXXI. The following comments may be made regarding these results.

1. Associations between _in vitro_ results and those for voluntary feed intake were not as close as in Series 1. Significant correlation between the results obtained by the _in vitro_ technique

TABLE XXX

ANALYSIS OF FORAGES USED IN SERIES 2
Experiment 4

Forage Species	Crude Protein Content (Air-dry Basis)	Voluntary Dry Matter Intake (Air-dry Basis)
	%	g/day
Red Clover	15	1516
Trefoil	14	1422
Alfalfa	16	1094
Brome Grass	8	1015
Timothy	7	786

TABLE XXXI

DRY MATTER LOSS AND FATTY ACID PRODUCTION FOR FORAGES IN SERIES 2 -- Experiment 4

Forage Species	Percent Dry Matter Loss		Fatty Acid Production mg. per 500 mg. forage	
	24 hrs.	48 hrs.	24 hrs.	48 hrs.
Red Clover	40.8	56.5	78.1	129.2
Trefoil	32.5	47.6	71.5	112.8
Alfalfa	36.2	50.6	80.2	123.6
Brome Grass	26.8	44.8	55.4	96.4
Timothy	23.8	38.6	51.6	80.4

and voluntary feed ingestion was not demonstrable.

2. The high loss of dry matter and yield of fatty acid compared to the low voluntary intake for the alfalfa sample seem to be the source of most of the variation from the results expected on the basis of the data from Series 1.

3. The values for dry matter loss and fatty acid

production in vitro as well as for voluntary feed intake in vivo for forages of the same species are considerably higher in Series 2 than they are in Series 1.

Discussion: The rank order of forages in Series 1 for yield of volatile fatty acid is exactly the same as that for voluntary consumption while the rank order of forages for loss of dry matter is slightly different. This suggests that the fatty acid production may be more reliable than the dry matter loss as an index of the voluntary intake of a forage.

The behavior of individual forages in Series 1, especially trefoil and red clover, seems to offer evidence for interesting speculation. The high values for the dry matter loss of red clover as compared to the yields of fatty acid from this forage suggest that factors other than the microbiological fermentation are involved in the dry matter loss values. The latter values do not distinguish between soluble dry matter present in feeds as they are placed in the fermentation sacs and that released by fermentation. Thus, for forages of different soluble dry matter content, the artificial rumen technique employed may not give an accurate picture of the amount of dry matter released by fermentation. The higher loss of dry matter of the red clover compared to that of trefoil may simply indicate that the latter sample contained less of soluble nutrients than did the former.

Since the main end product of the fermentation of cellulose and hemicellulose by rumen bacteria is volatile fatty acid, it would seem logical to assume that fatty acid production is a reasonably accurate index of the extent of this fermentation. Therefore, the fact that the trefoil sample gave the highest yield of

fatty acid suggests that the cellulose fraction of this forage was more completely fermented than was that of the red clover sample.

In this series the voluntary consumption of trefoil was higher than that of any other forage. In the light of this observation, the evidence that the cellulose fraction of trefoil seems to be more rapidly fermented than that of red clover, even though the latter forage appears to contain more soluble nutrients, lends support to the proposal of Crampton (1957) that feed intake under conditions of *ad libitum* feeding depends to a great extent on the rate of digestion of the cellulose and hemicellulose of the forage.

The results obtained in Series 2 are not in full agreement with those of Series 1. This is mainly because the dry matter loss and fatty acid production of the alfalfa sample are much higher than might be expected on the basis of the voluntary intake of this forage. These data for the *in vitro* fermentation indicate that digestion of the fiber fraction of the alfalfa sample is almost as thorough as is that of the red clover sample yet voluntary feed intake of alfalfa is almost as low as that of the brome grass. This suggests that factors other than rate of digestion of cellulose are involved in determining the amount of a forage an animal will voluntarily consume.

Whether the bloat-producing tendencies of this sample of high quality alfalfa fed as the sole diet of sheep had some effect is not known. The shorter feeding period of Series 2 may have affected consumption, and palatability may also have been a factor in lowering the consumption of this sample of forage, but there is no evidence to indicate that this might be so. The high

protein content of this alfalfa sample may have affected fermentations in vitro but not in vivo, but only more extensive investigation could demonstrate this.

In comparing results in vitro and in vivo for Series 1 and Series 2 it is interesting to note the increased dry matter loss, fatty acid production and voluntary feed intake of the artificially cured forages in Series 2. For example, red clover in Series 1 yielded 108.7 mg. of fatty acid per 500 mg. forage after 48 hours of fermentation, while the red clover in Series 2 yielded 129.2 milligrams. The voluntary consumption of red clover in Series 1 was 1070 g. per day and in Series 2 was 1516 g. per day. While other factors, such as stage of maturity could be involved, it seems highly probably that the method of handling the forages in Series 2, which reduced possibilities of leaching, shattering and fermentation during curing, resulted in increased nutritive value of the forages. The miniature artificial rumen techniques might be of value in investigating the effects of methods of curing forage. Several forages, cured under different conditions and by different methods could be assayed using the artificial rumen technique to determine the relative quality of the forages.

The main limitation of these trials was the lack of sufficient numbers of samples to establish a definite trend. Further investigation seems to be justified.

Summary:

1. Two series of 5 forages were fermented in the miniature artificial rumen and the dry matter loss and the fatty acid production were determined.

2. In Series 1 a high degree of association was demonstrated between the voluntary feed consumption and the artificial rumen fermentation as measured by the volatile fatty acid production and the loss of dry matter. The results of the fermentations of Series 2 were not in complete agreement with those of Series 1.
3. The volatile fatty acid production seemed to give a more accurate estimate of voluntary intake than did dry matter loss. It was suggested that one reason for this may be that fatty acid production is a better index of rate of cellulose digestion than is dry matter loss.
4. A comparison of the results obtained *in vitro* and *in vivo* in Series 1 and Series 2 suggested that artificial curing increased the quality of the forages.

EXPERIMENT 5

Introduction: The increased yields obtained when forage crops are correctly fertilized is often accompanied by increases in the proportions of nutrients in the crops. Reports by Wyatt (1945) and Newton et al. (1948) showed that the protein content of grains grown on sulfur deficient soils is increased by sulfur fertilization. Renner et al. (1953) showed that this increase in level of protein was accompanied by an increase in the proportions of 9 essential amino acids. Bentley et al. (1956) showed that the protein content of legume hay grown on such soils may be increased by as much as 46% as a result of fertilization, and that rabbits fed forages grown on sulfur deficient soils gained weight more rapidly and more efficiently when fed those roughages which had received sulfur-fertilization. In cooperation with Dr. C. F. Bentley and Mr. D. H. Laverty, Department of Soil Science, University of Alberta, it was decided to extend to ruminants the studies of the effects of sulfur-fertilization of forage crops on the grey wooded soils of Alberta.

The objectives of Experiment 5 were:

1. to determine by means of feeding trials with rabbits and sheep whether differences could be demonstrated between the nutritive values of forages grown on fertilized and non-fertilized plots, and,

2. to assess the efficacy of the miniature artificial rumen in the detection of differences in the nutritive value of these forages.

Description of Forages: All forages were samples of alsike clover grown on sulfur deficient soil near Breton, Alberta. One sample was from an unfertilized control plot and 4 samples were from plots fertilized as follows:

1.	N.S.	Ammonium Sulfate	(21-0-0)	@ 80 lbs. per acre
		Ammonium Nitrate	(33.5-0-0)	@ 190 lbs. per acre
2.	N.P.S.	Ammonium Sulfate	(21-0-0)	@ 80 lbs. per acre
		Ammonium Nitrate	(33.5-0-0)	@ 190 lbs. per acre
		Ammonium Phosphate	(11-48-0)	@ 125 lbs. per acre
3.	N.P.K.S.	Ammonium Sulfate	(21-0-0)	@ 80 lbs. per acre
		Ammonium Nitrate	(33.5-0-0)	@ 120 lbs. per acre
		Ammonium Phosphate	(11-48-0)	@ 125 lbs. per acre
		Potassium Chloride		@ 65 lbs. per acre
4.	P.S.	Ammonium Sulfate	(21-0-0)	@ 150 lbs. per acre
		Super Phosphate	(0-20-0)	@ 150 lbs. per acre

The forages were wilted in the field and dried indoors. They were ground and pelleted. For analysis aliquot samples of the pelleted material were ground in a Wiley Mill through a 40 mesh sieve and stored under refrigeration. Table XXXII gives the analysis of these feeds as supplied by the Department of Soil Science, University of Alberta.

Experimental: Rabbit and sheep trials were conducted by Mr. D. H. Laverty, Department of Soil Science. Albino rabbits of 6 to 9 weeks of age were fed a commercial rabbit feed for 10 days and the gains in weight were recorded. They were then allotted to groups according to litter, weight and gain in weight during the preliminary period. Each group of 3 rabbits was then fed one of the

5 feeds for a period of 10 days. Feed consumption was recorded and the feces were collected. The consumption and digestibility of dry matter and energy were determined.

Four Suffolk shearling rams were used as experimental animals for the sheep trials. These animals were wormed and sheared just before going on trial. Each sheep was fitted with a canvas feces bag and was allotted at random to 1 of 4 feeds. The feed from the phosphorus-sulfur plot was not used because there was not enough material available for feeding trials. The animals were fed as much as they would voluntarily consume. After a preliminary period of 7 days, a digestibility trial was conducted for 8 days during which feces were collected and weighed. At the end of the digestibility trial, the sheep were maintained on the test feeds for an additional 15 days, making the total feeding period 30 days. There was not sufficient feed to hold the animals on the trial for a longer period. Voluntary feed consumption was recorded daily for the 30-day period and the digestibility of energy and dry matter were determined for the 8-day digestibility trial.

The dry matter loss and volatile fatty acid production in the artificial rumen were determined for each of the 5 forages in duplicate. A washed random inoculum was used.

Results and Discussion: The results of the rabbit trials are given in Table XXXIII and those of the sheep trials in Table XXXIV. The data for dry matter loss and volatile fatty acid production in the artificial rumen are presented in Table XXXV.

The rabbit trials, sheep trials and artificial rumen assays all indicate that the feeding value of forages grown on a

TABLE XXXII

PARTIAL ANALYSIS OF FORAGES -- Experiment 5

Treatment	% Nitrogen	% Crude Fiber	Calories/gram
Control	2.28	22.9	3.88
N S	3.28	22.4	3.98
N P S	3.42	21.1	4.00
N P K S	3.34	22.0	3.91
P S	3.30	20.6	4.07

TABLE XXXIII

RESULTS OF RABBIT FEEDING TRIALS -- Experiment 5

Treatment	Feed Consumed Per Day g.	Digested Dry Matter %	Digested Energy %	Apparent Digestible Energy Per Day Cal.	Gain g.
Control	80.4	54.7	49.3	154	-133
N S	183.5	58.7	54.0	395	397
N P S	168.0	60.8	55.4	372	322
N P K S	157.5	62.1	55.9	345	397
P S	146.5	61.8	57.6	343	320

TABLE XXXIV

RESULTS OF SHEEP FEEDING TRIALS -- Experiment 5

	Feeding Trial			Digestion Trial	
Treatment	Feed Consumed Per Day g.	Apparent Digestible Energy Per Day Cal.	Gain lbs.	Digested Dry Matter %	Digested Energy %
Control	2,340	5,030	-6	58.8	55.4
N S	3,780	8,870	5	61.4	59.0
N P S	3,530	8,700	10	64.2	61.7
N P K S	3,280	8,270	0	67.1	64.4

TABLE XXXV

RESULTS OF ARTIFICIAL RUMEN TRIALS -- Experiment 5

Treatment	% Dry Matter Loss		mg. per 500 mg. forage Fatty Acid Production	
	24 hrs.	48 hrs.	24 hrs.	48 hrs.
Control	36.9	47.8	60.4	93.6
N S	42.2	58.4	73.4	120.3
N P S	44.1	63.4	73.5	132.8
N P K S	44.8	59.8	75.2	118.3
P S	44.6	56.5	83.0	124.9

sulfur-deficient grey wooded soil near Breton, Alberta is markedly increased by sulfur fertilization. An experiment of different design would be necessary to assess the effects and interactions of nitrogen, phosphorus, and potassium. In this experiment only the effect of sulfur fertilization is demonstrated.

The feeding trials have several limitations. The use of rabbits as test animals in estimating the value of forages for ruminants is not entirely valid because the rabbits utilize cellulose through cecal fermentation and practice coprophagy. This process is not analagous to rumen fermentation and the rumination cycle of ruminants. However, if the limitations in the use of rabbits are recognized and considered, these animals may be used profitably in pilot experiments.

The main limitation of the sheep experiment was that only one animal was used per feed because of the limited amount of forage. Results of one trial without replication cannot be regarded as accurate as there is no way of determining the error involved in individual variation between test animals.

The sheep in the digestibility trials were fed all the forage they would voluntarily consume in order to obtain data for voluntary feed intake in connection with the hypothesis presented in Experiment 4. The usual procedure in trials conducted to determine the digestibility of feeds is to limit the feed intake of all animals to levels at or near maintenance in order to minimize differences attributable to varied intake. The extent of the effect of the varying levels of intake on the digestibility of these forages is not known. It may, however, be a source of some error.

Despite the limitations of these two feeding trials and those inherent in the artificial rumen technique, all these methods indicate the same trend so that the results of each trial support those of the others. On the basis of all three assays of forage quality, it would be reasonable to assume that the indications that fertilization under these circumstances increased the feeding value of forages are reliable.

Summary:

1. Rabbit feeding trials, sheep feeding trials and miniature artificial rumen measurements were used to determine whether fertilization of forage crops grown on plots of sulfur-deficient grey wooded soil would increase the feeding value of the forage.
2. In this case, forages from plots fertilized with sulfur were superior to those from the unfertilized plots in the dry matter and energy consumed by test animals as well as in nitrogen content. In most cases animals fed the fertilized roughages gained weight while those fed unfertilized

roughages lost weight.

3. Volatile fatty acid production and dry matter loss, as determined by the artificial rumen technique, were greater for samples from fertilized plots than for the samples from the check plot.
4. While there are limitations in the methods used, it is felt that the evidence for the effects of sulfur fertilization on forage quality under the described conditions is relatively reliable.

CONCLUSIONS

Results obtainable by the use of the miniature artificial rumen are closely associated with several criteria which are used to estimate the feeding value of forages. These include digestibility of dry matter, crude and digestible protein content, voluntary feed consumption and perhaps several others. It cannot be said that results of *in vitro* fermentation may be used as indices of any of these factors because their degrees of association with them have limitations and anomalies. It is probable that all these phenomena are related and represent different facets in the understanding of the complex composition of a forage and the even more complex relationships between that forage and the animal which eats it.

Data from artificial rumen studies at best represent an approximation of the extent and nature of bacterial fermentation in the intact rumen. This fermentation is an important factor in roughage utilization and, to a greater or lesser extent, influences most of the available indices of feed value. The fermentation itself is influenced by a variety of factors in the forage and in the animal. Each different forage largely controls the fermentation of itself by the nutrients it supplies to the rumen microflora, and the animal influences the fermentation by the type and amount of roughage it consumes.

Although some generalizations have been made in order to indicate trends which may exist, any attempt to categorize the associations between the various factors considered is dangerous from both a practical and a theoretical viewpoint. To say that an association exists is, in many cases, valid, but to attempt to

assign cause and effect on the basis of the evidence presented in this thesis is not justified.

Certain relationships exist between various accepted indices of feed quality and the results obtained using the miniature artificial rumen. On the basis of these associations it is often possible to assess with reasonable accuracy the nutrient value of a forage *in vivo* by using results obtained *in vitro*. Moreover, the miniature artificial rumen may be very useful in investigations into the many and complex phenomena connected with the utilization of high-fiber feeds by ruminant animals.

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